EXHIBIT 2, Part 2

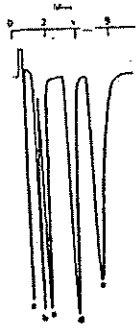


Figure 6. IPC of several anions: (a) carbonate, 1.8 μg; (b) chloride, 1.4 μg; (c) phosphate, 3.8 μg; (d) azide, 5.0 μg; (e) nitrate, 10 μg.

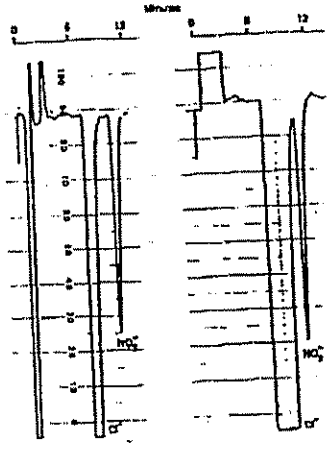


Figure 7. Determination of nitrite in chloride: (A) 58.5 ppm chloride, 4.6 ppm nitrite; (B) 585 ppm chloride, 4.6 ppm nitrite.

Figures 7 and 8 show the determination of nitrite in chloride and of traces of sulfate in a high background of salt. Both illustrate the excellent selectivity and sensitivity of which IPC is capable.

As discussed earlier, sensitivity may be improved by decreasing eluent concentration. An example of this is provided in Figure 9. The fairly potent sulfobenzoate ion was chosen as the displacing species so that relatively low concentrations would suffice to elute the sample ion, in this case sulfate, with reasonable speed. An appropriately low capacity column was also used. The trough due to sulfate and showing good signal to noise resulted from a 100-μL injection of 10^{-6} M sodium sulfate. The detectability of sulfate under these conditions is approximately 1 ng, attesting to the high sensitivity attainable by the technique.

IPC has exceptional capabilities for handling ionic species with high affinity for anion exchangers. Noteworthy is the rapid elution of the polyphosphate species (Figure 10), an ion that is normally very difficult to displace.

Cation Separations. A number of cation separation schemes were developed by using the IPC approach. Figure 11 illustrates a rapid separation of sodium, ammonium, and potassium. This separation is noteworthy in that it was obtained on a very small column (2.8 × 20 mm) of high specific capacity cation exchanger. The eluent was copper sulfate (0.01 M), copper being the UV-absorbing displacing ion. A column and eluent such as this have been used to determine small amounts of sodium and potassium in concentrated (20%) calcium chloride solution. Only moderate dilution of the sample is required in view of the high capacity of the resin employed. After the monovalent ions had been eluted, the resin was flushed briefly with a concentrated (1 M) solution of copper nitrate in order to displace divalent ions which would otherwise have appeared at a much later time and interfered with subsequent chromatograms. We found it convenient to introduce this purge solution by way of another sample injection valve equipped with a large (0.5 mL) loop. A separation of sodium, potassium, calcium, and magnesium (Figure 12) was achieved by using a split column technique wherein two columns of equal length but containing resins of different specific capacities were connected in series and appropriately switched.

Joint Anion and Cation Determination. We have demonstrated that indirect photometric chromatography may be

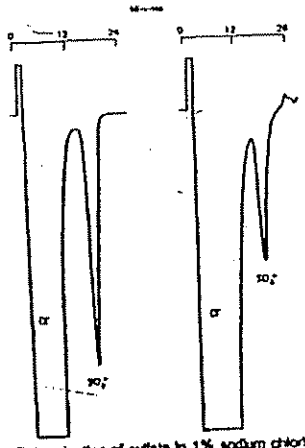


Figure 8. Determination of sulfate in 1% sodium chloride: (A) 100 ppm sulfate; (B) 10 ppm sulfate.

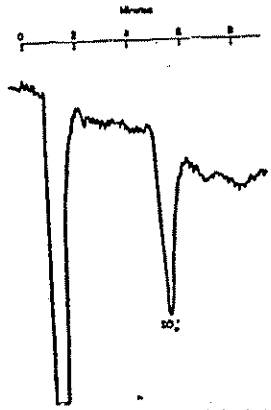


Figure 9. Sensitivity of IPC. Sulfate peak due to a 0.1 mL injection of 10^{-6} M sodium sulfate.

Table III. Details on Chromatographic Conditions in Various Separations

Figure 2 column: 4 × 250 mm, SAR-40-0.6
eluent: 10^{-3} M sodium phthalate, pH 7–8
flow rate: 2 mL/min; sample size 0.02 mL
detector: LDC 1203, 0.032 AUFS
sample: 2×10^{-3} M in each of chloride and nitrite; 5×10^{-3} M in each of bromide, nitrate, and sulfate

Figure 6 column: 4 × 250 mm, SAR-40-0.6
eluent: 10^{-3} M sodium phthalate, 10^{-3} M boric acid, pH 10
flow rate: 5 mL/min; sample vol 0.02 mL
detector: Perkin-Elmer LC-75, 0.32 AUFS

Figure 7 column: 4 × 250 mm, SAR-40-0.6
eluent: 10^{-3} M sodium phthalate, 10^{-3} M boric acid, pH 9
flow rate: 2 mL/min; sample vol 0.02 mL
detector: LDC 1203, 0.32 AUFS

Figure 8 column: 4 × 250 mm, SAR-40-0.6
eluent: 10^{-3} M sodium phthalate, 10^{-3} M boric acid, pH 9
flow rate: 2.0 mL/min; sample vol 0.1 mL
detector: LDC 1203
sensitivity: (A) 0.016 AUFS; (B) 0.004 AUFS

Figure 9 eluent: 10^{-4} M sodium sulfobenzoate pH 8
flow rate: 1 mL/min
detector: Varian UV-50 set at 224 nm
sensitivity: 0.005 AUFS

Figure 10 column: 2.8 × 250 nm, SAR-40-0.6
eluent: 10^{-3} M sodium trimetate, pH 8
flow rate: 1 mL/min; sample size 0.02 mL
sample: 2 g/L potassium pyrophosphate, 2 g/L sodium tripolyphosphate
detector: Varian UV-50 set at 296 nm
sensitivity: 0.02 AUFS

Figure 11 column: 2.8 × 20 mm, Dowex 50 resin
eluent: 0.01 N copper sulfate
flow rate: 0.7 mL/min; sample size 0.02 mL
sample: 0.01 N in each of sodium, ammonium, and potassium
detector: Varian UV-50 set at 252 nm
sensitivity: 0.05 AUFS

Figure 12 first column: 2.8 × 250 mm surface sulfonated styrene–DVB 0.015 mequiv/g
second column: 2.8 × 250 mm surface sulfonated styrene–DVB 0.087 mequiv/g
eluent: 1.25×10^{-3} M copper sulfate
detector: Perkin-Elmer LC-75 set at 215 nm

Figure 13 columns: 4.6 × 250 mm Partisil SAX followed by 4.6 × 250 mm Partisil SCX
eluent: 5×10^{-3} M copper nitrate
detector: Perkin-Elmer LC-75 set at 241 nm
sample: 0.02 mL; 0.2 M NaF, 0.2 M RbCl, 0.1 M $MnCl_2$

extended to simultaneous joint anion and cation analysis by combining chromophoric anion and cation mobile phase ions with suitable ion exchange columns. For extension of IPC to combined analysis of anions and cations in a single chromatograph, a special eluent must be chosen. In accordance with the principles already outlined, the eluent for analysis of, say, only anions in a sample has a 2-fold function: to displace anion bands individually from the column and to render them detectable as transparencies in contrast to eluent anion UV absorbance. Joint analysis of anions and cations with a single eluent and UV detection, then, requires mobile phase anion and cation both with UV absorbance and appropriate sample elution power. A necessary further consideration is that mobile phase anion and cation each contribute approximately equally to the absorbance at base line since we have seen already how sensitivity to eluting sample ions relates to mobile phase absorbance.

Taking the various factors into account, we found copper nitrate to be a suitable eluent. As determined by a Cary 15 spectrophotometer at 241 nm, $\epsilon_{Cu} = 37.5$ L equiv^{-1} cm^{-1} and $\epsilon_{NO_3} = 77.5$ L equiv^{-1} cm^{-1}. It follows that a 5×10^{-3} M $Cu(NO_3)_2$ solution would produce a base line absorbance of

$$A_{TOT} = A_{Cu} + A_{NO_3} = (\epsilon_{Cu} + \epsilon_{NO_3}) \times \text{normality} \times \text{pathlength}$$

and so

$$A_{TOT} = 1.15$$

in a detector with a 1-cm pathlength. This is an acceptable base line absorbance value and thus a 5×10^{-3} M copper nitrate eluent was selected along with a detection wavelength of 241 nm.

The separating columns chosen were commercially available strong anion and cation exchange columns arranged in series. The cation exchanger was 4.6 × 250 mm Partisil 10-SCX from Whatman containing 10-μm microparticulate packing with siloxane-bonded sulfonic acid exchange groups. The anion exchanger was a 4.6 × 250 mm Partisil 10-SAX with 10-μm microparticulate packing and siloxane-bonded quaternary anion exchange sites.

The chromatogram obtained from an injection of a synthetic mixture comprising 0.2 M NaF, 0.2 M RbCl, and 0.1 M $MnCl_2$ is shown in Figure 13. The first three troughs are the deficiencies in the copper absorbance caused by the emergence of the three sample cations, the nitrate absorbance (concentration) remaining constant within this region. The last two troughs are the deficiencies in nitrate absorbance due to sample anions while the copper absorbance remains constant.

Calibration. Calibration runs for the three ions, sulfate, nitrate, and phosphate yielded curves that indicate a convenient linear dependence of trough depth on the amount of ion injected.

There is an interesting aspect to calibration in the IPC mode in that for many ions the area of the trough is not dependent on the ion injected but only on its amount. This is a natural result of the method of monitoring since each equivalent of sample ion displaces the same amount of monitoring ion from the mobile phase irrespective of the sample ion. To demonstrate this, separate injections of accurate amounts of nitrate, sulfate and phosphate were eluted by sodium phthalate (pH 8) and the areas of the troughs measured. The results are shown in Table IV.

The area of trough per equivalent of ion is indeed approximately independent of the ion injected—for these three ions. On the basis of this observation we expect anions to adhere to this rule. Anions of acids with medium to high pK's should give responses determined by their valence at the ambient pH of the eluent. Phosphate, for example, exists predominantly as the HPO_4^{2-} species at pH 8 so that 1 mol

Figure 13. Separation of pyro- (PP) and tripoly- (TPP) phosphates.

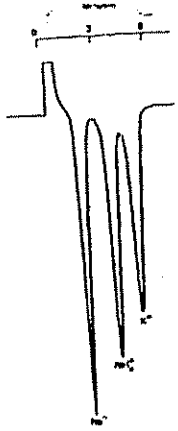


Figure 11. IPC of cations.

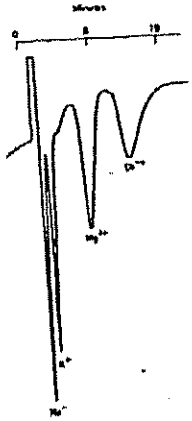


Figure 12. Separation of mono- and divalent cations by a split column technique.

Table IV. Calibration Data for Nitrate, Sulfate, and Phosphate

Injection	area of trough (arbitrary units)	area of trough/(mequiv/L) of ion injected
5×10^{-5} M sodium nitrate	117.5	23.5
2.5×10^{-5} M sodium sulfate	111.0	22.2
1.67×10^{-5} M sodium orthophosphate	80.4	24.1

of phosphate injected would be expected to displace 2 equiv of monoion. The data of Table IV support this expectation.

CONCLUSIONS

This work has demonstrated that photometers may be used as chromatographic detectors for accurate, sensitive determination of transparent ionic species commonly considered photometrically undetectable. The technique, indirect photometric chromatography as we have called it, shows considerable potential in the area of inorganic and organic ion analysis.

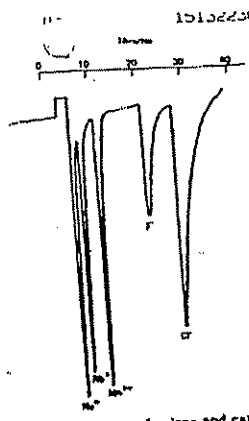


Figure 13. Joint determination of anions and cations by IPC.

In IPC, detection of the eluting sample is accomplished by monitoring a change in property of the column effluent as the eluent ion is displaced by the sample ions. Clearly, sensitivity in such a case is impaired when the eluent and the sample ions possess this property to a comparable extent. Such is the case with suppressorless conductometric monitoring—both sample ion and eluent ion are conducting and the closer the values of their equivalent conductances the poorer the sensitivity for that sample ion. In IPC on the other hand one chooses a monitoring wavelength where the displacing ion is absorbing and the sample ion is not, hence satisfying a condition for obtaining maximum sensitivity. This we claim places IPC in a superior position among suppressorless ion chromatographic methods.

Indirect photometric chromatography is a promising new approach to a number of ion analysis problems.

ACKNOWLEDGMENT

The authors have appreciated the experimental assistance of D. F. Scheddel during the course of this work.

LITERATURE CITED

(1) Small, H.; Stevens, T. S.; Bauman, W. C. *Anal. Chem.* 1975, *47*, 1801–1809.
(2) Small, H. In "Applications of Ion Chromatography in Trace Analysis"; Lawrence, J. F., Ed.; Academic Press: New York, 1981.
(3) Stevens, T. S.; Davis, J. C.; Small, H. *Anal. Chem.* 1981, *53*, 1488–1492.
(4) Gjerde, D. T.; Fritz, J. S. *J. Chromatogr.* 1979, *176*, 199–206.
(5) Gjerde, D. T.; Fritz, J. S.; Schmuckler, G. *J. Chromatogr.* 1979, *186*, 509–519.
(6) Gjerde, D. T.; Schmuckler, G.; Fritz, J. S. *J. Chromatogr.* 1980, *187*, 35–45.
(7) Fritz, J. S.; Gjerde, D. T.; Becker, R. M. *Anal. Chem.* 1980, *52*, 1519–1522.
(8) Pohl, C. A.; Johnson, E. L. *J. Chromatogr. Sci.* 1980, *18*, 442–452.
(9) Laurent, A.; Bourdon, R. *Ann. Pharm. Fr.* 1978, *36* (9–10), 453–460.
(10) Ringbom, A. "Complexation in Analytical Chemistry"; Interscience: New York, 1963; pp 198–199.
(11) Kolthoff, I. M.; Sandell, E. B. "Textbook of Quantitative Inorganic Analysis"; Macmillan: New York, 1949; pp 663–668.
(12) Small, H.; Stevens, T. S. U.S. Patent 4 101 460, July 18, 1978.
(13) Stevens, T. S.; Small, H. *J. Liq. Chromatogr.* 1978, *1* (2), 123–132.
(14) Small, H.; Ed.; Proceedings of an International Conference on "The Theory and Practice of Ion Exchange"; Street, M., Ed.; The Society of Chemical Industry: London, 1976; pp 32–1 to 32–10.

Received for review September 16, 1981. Accepted November 6, 1981. The methods described in this publication are the subject of pending patents which are licensed to Dionex Corporation for commercial use.

Journal of Chromatography, 470 (1989) 299–308
Elsevier Science Publishers B V., Amsterdam — Printed in The Netherlands

CHROM. 21 384

INDIRECT PHOTOMETRIC DETECTION IN CAPILLARY ZONE ELECTROPHORESIS

F. FORET[a], S. FANALI and L. OSSICINI
Istituto di Cromatographia CNR, Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo, Rome (Italy)
and
P. BOČEK*
Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Leninova 82, 611 42 Brno (Czechoslovakia)
(First received January 9th, 1989; revised manuscript received January 23rd, 1989)

SUMMARY

An indirect photometric detection method is described which is based on the use of an absorbing co-ion as the principal component of the background electrolyte. The zones of non-absorbing ionic species are revealed by changes in light absorption due to charge displacement of the absorbing co-ion. Theoretical considerations are given for selecting a suitable absorbing co-ion to achieve a high sensitivity of detection.

The role of electromigration dispersion is illustrated by experiments and the effects of the differences in the effective mobilities of sample ions and that of the absorbing co-ion are discussed. The highest sensitivity can be achieved for sample ions having an effective mobility close to the mobility of the absorbing co-ion. In such a case, the concentration of the sample component in its migrating zone can be high while electromigration dispersion is still negligible. The useful dynamic range of the detection is then limited by the linearity and noise of the detector, the former parameter being given mostly by the shape of the on-column detection cell. The best sensitivities can be obtained in low-concentration background electrolytes containing a co-ion with high absorption at a given detection wavelength.

It is shown that indirect photometric detection can be useful for detecting substances that have no optical absorption in the UV and/or visible region, provided that the composition of the background electrolyte is selected correctly.

INTRODUCTION

Sensitive and reliable universal detection of all migrating zones in capillary zone electrophoresis (CZE) is of key importance for the utilization of this technique in practice.

* Permanent address: Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Leninova 82, 611 42 Brno, Czechoslovakia.

0021-9673/89/$03.50

In capillary isotachophoresis[1], potential gradient and conductivity detectors are currently used for this purpose. In CZE[2], the use of potential gradient[3] and conductivity[4–6] detectors is limited to ionic species that have an effective mobility that is substantially different to that of the background electrolyte co-ion. When this difference decreases, the detector signal also decreases and it can often be masked by the noise generated by electrochemical reactions on the sensing electrodes of the detector cell[4]. Therefore, selective optical detectors are currently used here in either the absorbance[7] or the fluorescence mode[8]. Promising results were reported also with electrochemical[9] and mass spectrometric[10] detection. The possibility of using a selective optical detection system as a universal detector is offered by the utilization of the indirect photometric mode. Indirect photometric detection has already been well adopted in ion chromatography[11,12] and, by monitoring the counter ion, it has also been used as a universal detector in isotachophoresis[13].

In CZE, indirect fluorescence detection has been used to monitor the migration of zones of some amino acids[14], nucleotides, iodate, hydrogencarbonate and lysozyme[15]. The reported detection limits are impressive, mainly owing to the high intensity of the excitation laser beam and small inner diameter (15 μm) of the separation capillary used. In this paper we propose a method for the universal indirect detection of zones in CZE based on absorption photometric monitoring of a suitable absorbing co-ion which is the principal component of the background electrolyte.

THEORETICAL

The excellent separation properties of CZE are due mainly to the low dispersive performance of the equipment. In the optimum limiting case, the dispersion of the migrating zones is determined only by diffusion, initial sample pulse width and Joule heat[16,17]. In practice, however, dispersion due to sorption phenomena[17–19] and electromigration dispersion[4,20] contributes significantly to the dispersion of migrating zones. The latter type of dispersion is closely related to the detection. It always occurs during the migration of sample ions which possess effective mobilities different to that of the background electrolyte co-ion; the higher the concentration of the sample component in its zone, the more pronounced is the electromigration dispersion. The electromigration dispersion is different for different ions, and the method of suppressing it is to keep the solute concentrations in their zones sufficiently lower than the concentration of the background electrolyte (BGE).

Obviously, the supprssion of electromigration dispersion by lowering the concentration of the solutes in their zones places greater demands on the detection sensitivity and limits the useful concentration range of detection in CZE. Generally, electromigration dispersion is considered to be negligible when the concentration of the solute ions is two orders of magnitude lower than that of the BGE co-ion[4,18].

The absorbance detectors currently used in CZE exhibit a noise level of *ca.* $1 \cdot 10^{-4}$ absorbance units (A.U.) and their useful dynamic concentration range covers roughly three orders of magitude, as the upper limit of linearity is *ca.* 0.1 A.U. This upper limit is given mainly by the shape of the on-column detector cell, which is exclusively of circular cross-section in present practice. Here the Lambert–Beer law does not hold true in the form derived for a cell with plane parallel windows.

The situation is shown schematically in Fig. 1. The light beams delimited by the

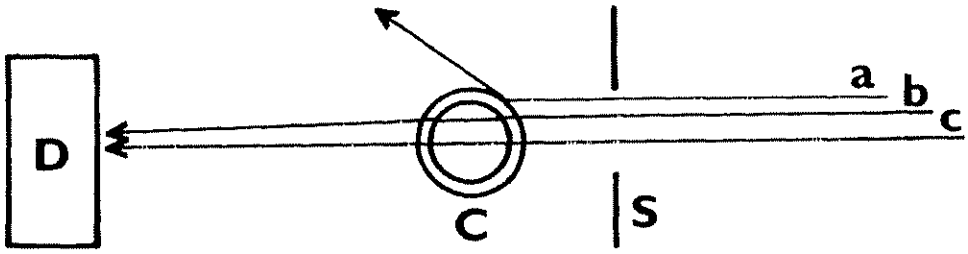


Fig. 1. On-column absorbance detection in capillaries. For details, see text.

slit S strike the capillary C at different positions. The part of the radiation denoted by a is refracted by the wall of the capillary and is lost. The beams b and c pass through the solution inside the capillary and serve for the detection. Their path lengths are, however, different. Hence, for each beam i a special equation can be written in the form

$$I_i = I_{0i} \cdot 10^{-\varepsilon c d_i} \tag{1}$$

where I_i is the intensity of the ith beam, ε is the molar absorption coefficient of a sample of concentration c and d_i is the optical path length of the ith beam. To obtain explicitly the mean intensity of the radiation detected by the detector D, integration is necessary and the resulting absorbance is not a linear function of the concentration c. A more detailed numerical treatment of this problem can be found in the literature[21]. However, for low values of the exponent $\varepsilon c d_i$, eqn. 1 can be expanded into a series and, by neglecting higher terms, it can be derived that for the intensity I detected by the detector

$$I = I_0 (1 - 2.3\ \varepsilon c \bar{d}) \tag{2}$$

where $\bar{d}$ is the mean optical path length in the capillary ($\bar{d} \approx 0.6$ I.D.). Eqn. 2 can be used in practice to describe the attenuation of the light beams up to *ca.* 0.1 A.U. At higher absorbance, the detected and registered peaks are already significantly distorted owing to the non-linearity of the detector.

When considering a capillary of I.D. 100 μm filled with a solution of a solute having $\varepsilon = 10\,000$ l mol^{-1} cm^{-1}, the corresponding admissible maximum concentration is about 10^{-3} M. It is interesting that according to eqn. 2, the detector to be used in capillary techniques does not need to be equipped with a logarithmic converter.

Returning to the mutual relationship between the detection linearity and electromigration dispersion, it follows that if indirect photometry is to be used for detection in CZE, and if simultaneously the concentrations of solutes in the zones should be 100 times lower than that of the BGE co-ion, then the useful dynamic decrease in the BGE absorbance due to the migration of a zone (useful signal) would be only 0.001 A.U. When using a photometer with a noise level of 0.0001 A.U. the resulting signal-to-noise ratio is only 10, which is too low for practical use. Hence, another means of suppressing electromigration dispersion must be found which is based on the selection of a co-ion with a mobility close to those of sample components.

In such a case, the electromigration broadening of zones during the migration is negligible even if the concentrations of solutes reach the concentration of the BGE co-ion. It should be stressed here that this method of suppression of the electromigration dispersion is of key importance as it is advantageous both for detection (direct or indirect) and for achieving high separation efficiencies (number of theoretical plates). Concerning the selection of the counter ion of the BGE, species of low mobility should be selected to ensure a low conductivity of the BGE and hence prevent excessive Joule heating during the analysis.

Another practical hint concerns the sample injection. It is convenient to inject a low-concentration sample which is not mixed with the BGE[4]. In this instance the concentration effect applies across the stationary boundary and the narrow sample pulse obtained facilitates an increase in both the sensitivity of detection and the separation power. Of course, the Joule heat limits the injection of low-concentration (low-conductivity) samples by possible overheating at the point of injection.

EXPERIMENTAL

Equipment

The experiments were carried out in fused-silica capillaries of 130 μm I.D. kindly supplied by Dr. Doupovec (Physical Institute, Slovak Academy of Sciences, Bratislava, Czechoslovakia) and of 100 μm I.D. deactivated fused-silica capillaries (Chrompac International, Middelburg, The Netherlands). One end of the capillary was connected to the electrode vessel via a block of Perspex, equipped with a Hamilton valve, enabling the capillary to be rinsed and filled after each analysis with the help of a syringe. The other end of the capillary served for sample introduction and was held in a mechanical moving arm for easy movement of the capillary orifice from the electrode vessel to the raised sample vial for hydrodynamic injection and back for the analysis. A laboratory-made power supply delivering up to 14 kV and 100 μA was used to drive the separation.

The zones separated in a 130 μm I.D. fused-silica capillary that was 46 cm long (42 cm to the detection cell) were detected by a single-beam UV detector from a Tachophor 2127 ITP analyser (LKB, Bromma, Sweden) with the aid of a previously described fibre-optic on-line detection cell[5].

In some experiments a Varian 2550 variable-wavelength detector was used. In this instance the original flow cell was replaced by a holder made of hard black PVC, which held the 100 μm I.D., 40 cm long (30 cm to the detector) capillary tightly in the optical path of the detector.

Chemicals and electrolytes

All chemicals were of analytical-reagent grade, supplied by Fluka (Buchs, Switzerland). Distilled water was deionized on a mixed-bed ion exchanger.

Two types of background electrolytes were used. The first contained 0.02 *M* benzoic acid as the UV-absorbing anion and was titrated with histidine to pH 6.2; 0.1% Triton X-100 was added to this BGE to suppress the electroosmotic flow. The second BGE contained a lower concentration of the anion with a higher UV absorbance and consisted of 0.007 *M* sorbic acid titrated with histidine to pH 6.2. The elimination of electroosmosis by the addition of Triton X-100 failed in this instance

and, therefore, no additive was used. In both instances the concentration of the BGE was selected so that its absorbance was the limit of the detector linearity.

All values of mobilities were taken from published isotachophoretic data[22].

RESULTS AND DISCUSSION

The ranges of linear response of the detectors used were determined by filling the capillary with standard solutions of benzoic acid. The plot of signal *vs.* concentration of benzoic acid for the LKB detector is shown in Fig. 2. It can be seen that undistorted peaks can be recorded for a detector signal up to 250 mV. The noise level was *ca.* 2 mV. Similar plots were obtained with the Varian 2550 detector. This double-beam detector is equipped with a logarithmic converter and the response was linear up to 0.08 A.U. The noise level was lower than 0.0001 A.U., which is an order of magnitude better performance than that of the single-beam LKB detector.

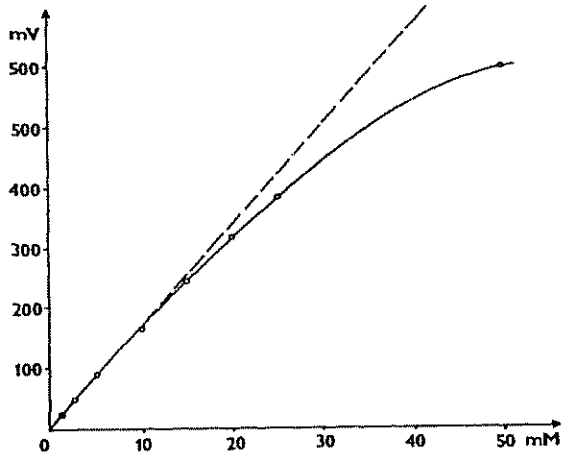


Fig. 2. Detector response *vs.* concentration of benzoic acid inside the 130 μm I.D. capillary.

In Fig. 3 the separation of fourteen model anions in BGE I with both indirect and direct photometric detection is shown. The model sample composition covers a wide mobility range from chloride (u = 79.08 · 10^{-5} $cm^2V^{-1}s^{-1}$) to glucuronate ($\bar{u}$ = 25.4 · 10^{-5} $cm^2V^{-1}s^{-1}$). The effective mobility of the UV-absorbing BGE co-ion (benzoate) is 32 · 10^{-5} $cm^2V^{-1}s^{-1}$ at this pH.

The electroosmotic flow was suppressed by the presence of 0.1% Triton X-100 in the BGE I and its magnitude was determined from the migration times of individual zones. The resulting electroosmotic mobility was low, being *ca.* 1 · 10^{-4} $cm^2V^{-1}s^{-1}$ in the cathodic direction.

As expected, highly mobile anions provide broad peaks with a diffuse front and sharp rear boundary and a low detector response. As the mobility of migrating anions decreases, the detector response increases and the peaks become narrower.

The best signal is obtained for the zone of hydroxyisobutyrate (HIBA), which

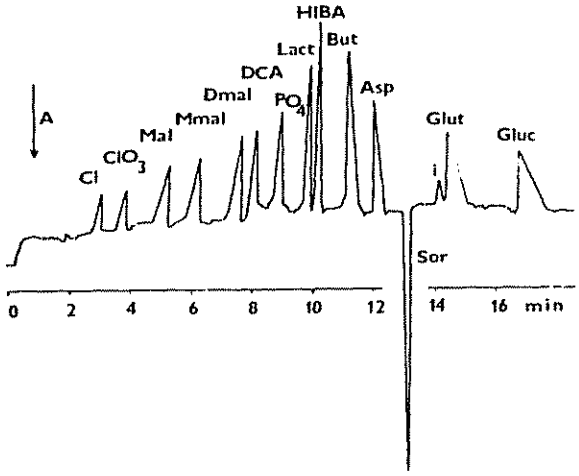


Fig. 3. CZE separation of anions in 130 μm I.D. capillary with indirect photometric detection with a modified LKB Tachophor detector operating at 254 nm. BGE I: 0.02 *M* benzoic acid–histidine at pH 6.2 + 0.1% Triton X-100. Driving current: 35 μA at 13 kV. Abbreviations: Mal = malonate; Mmal = methylmalonate; Dmal = dimethylmalonate; DCA = dichloroacetate; Lact = lactate; HIBA = hydroxyisobutyrate; But = butyrate; Asp = aspartate; Sor = sorbate; i = impurity; Glut = glutamate; Gluc = glucuronate.

has roughly the same effective mobility as benzoate. The slow ions form zones with a sharp front and diffuse rear boundary. Owing to the longer time of migration these zones are broader than the zones of fast ions.

As the noise of the LKB detector was *ca.* 0.001 A.U., a further increase in the sensitivity can be expected with the detector having lower noise. This is demonstrated by Fig. 4, where the separation of thirteen non-UV-absorbing ions was performed in a 100 μm I.D. capillary with the Varian 2550 spectrophotometric detector. Although a 3-fold lower concentration of the sample in comparison with previous experiments

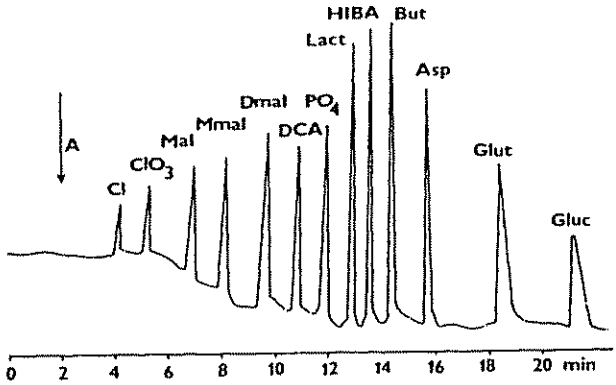


Fig. 4. CZE separation with indirect photometric detection using a Varian 2550 double-beam spectrometric detector operating at 254 nm. The 100 μm I.D. capillary was filled with BGE I. Driving current: 20 μA at 10 kV. For details, see text.

was injected here, a better signal-to-noise ratio was still obtained. On the other hand, a new problem arose here with the baseline drift, which was found to fluctuate with the BGE absorption owing to Joule heating during the analysis. For this reason the use of thinner capillaries seems to be advantageous, where theoretically also better separation efficiencies should be obtained[17,23].

Concerning the separation efficiency, it seems to be of interest to illustrate how it is limited by electromigration dispersion. In Fig. 5, the number of theoretical plates is plotted *vs* the difference between the mobility of the BGE co-ion and that of a sample species. Obviously, the number of theoretical plates reaches its maximum for ionic species having effective mobilities close to that of the co-ion. For greater differences in mobilities the separation efficiency decreases strongly.

Apparently, the use of BGE I provides satisfactory results, however, some conditions should be mentioned. The use of a relatively high concentration of BGE enables the electroosmosis to be reduced substantially by the simple addition of Triton X-100. On the other hand, the low absorbance of benzoate is the reason why the sensitivity of indirect detection is low in comparison with that of the direct detection of highly absorbing substances. This can result in peak masking even by trace UV-absorbing components in the sample. Such a situation is demonstrated in Fig. 6, where the original model mixture was enriched with *o*-aminobenzoate (OAB) and picrate at concentrations of $2 \cdot 10^{-4}$ *M*. Obviously the concentrations of both species added were three times lower than those of other sample components. It can be seen that the aspartate peak is partly overlaped by OAB and seems to be much sharper, and

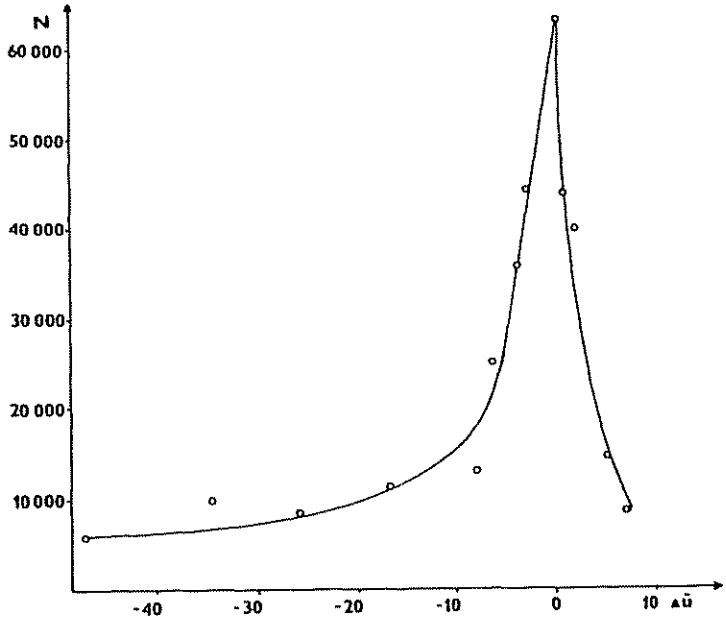


Fig. 5. Effect of electromigration dispersion on the separation efficiency. *N* is the number of theoretical plates, Δu is the difference $\tilde{u}_c - \tilde{u}_i$ (10^{-5}cm^{2}V^{-1}s^{-1}), where i and c are sample ionic species and co-ion, respectively

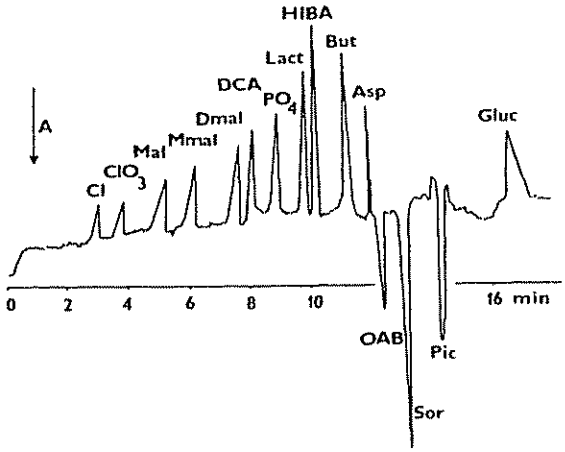


Fig. 6. Indirect photometric detection with peak masking by strongly absorbing trace components in the sample. The concentrations of *o*-aminobenzoate (OAB) and picrate (Pic) were $2 \cdot 10^{-4}$ *M*. The concentrations of other components were $6 \cdot 10^{-4}$ *M*. Other conditions as in Fig. 3.

the peak corresponding to glutamate disappeared completely owing to migration of picrate in the same zone.

The sensitivity of detection can be greatly increased by using a low-concentration but highly absorbing co-ion in BGE, namely, BGE II containing $7 \cdot 10^{-4}$ *M* sorbic acid. This solution has a low ionic strength and with the given instrumental arrangement strong cathodic electroosmotic flow was observed. The use of additives, *e.g.*, Triton X-100 did not suppress electroosmosis significantly. Therefore, no additives were used in further experiments and cathodic electroosmosis was utilizd to drive sample components through the detection cell. The separation of the model mixture of anions, driven cathodically by electroosmosis, is shown in Fig. 7.

The first detected positive peaks belong to potassium and lithium originating

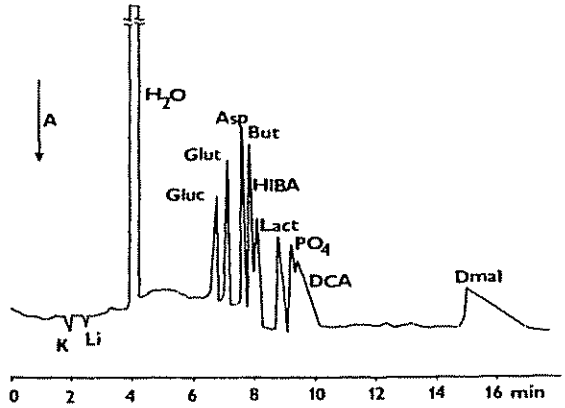


Fig. 7. CZE separation of anions with indirect photometry in low-concentration BGE II consisting of $7 \cdot 10^{-4}$ *M* sorbic acid–histidine at pH 6.2 with no additives. Driving current: 2 μA at 13 kV. LKB detector. For details, see text.

from the potassium phosphate and lithium lactate used for the sample preparation. The large rectangular peak of water transported by electroosmotic flow corresponds to the volume of sample injected and can be used as an electroosmotic marker.

The value of electroosmotic mobility, *i.e.*, the term $\varepsilon\zeta/\eta$, where ε, ζ and η are the permitivity of the BGE, the zeta potential and viscosity of the BGE, respectively, in the Helmholtz–Smoluchovski equation was found to be *ca.* $60 \cdot 10^{-5}$ $cm^2V^{-1}s^{-1}$. Obviously, the first peaks of anionic sample components correspond to low-mobility anions, whereas the highly mobile anions with electrophoretic velocities comparable to or higher than the electroosmotic velocity are not detected at all.

After the start of the analysis the sample ions migrate across the concentration boundary between the BGE and sample solution. As the sample concentration is low here ($2 \cdot 10^{-5}$ *M* of each ionic species), the migrating species are first concentrated across the above-mentioned boundary into a narrow sample zone in which their concentrations are adjusted to the values fulfilling the Kohlrausch regulation function[24]. Hence a sharp starting sample zone is created which aids positively the detection of sample components. During the following migration (superposition of electroosmosis and electrophoresis), however, zones containing ions with effective mobilities different from that of the sorbate ion are broadened by electromigration dispersion. The sharpest zones with the best detector response again provide anions with a mobility close to that of the BGE co-ion (sorbate).

The detection sensitivity in this system is roughly 50 times better than that in the previous instance and the detection limit even with a single-beam detector approaches 0.5 pmol injected.

Finally, we should mention an important practical aspect of the utilization of electroosmotic flow for driving the sample species along the separation path. The magnitude of the electroosmotic flow is strongly dependent on the history of the capillary used. This is demonstrated in Fig. 8, which shows the separation of a sample

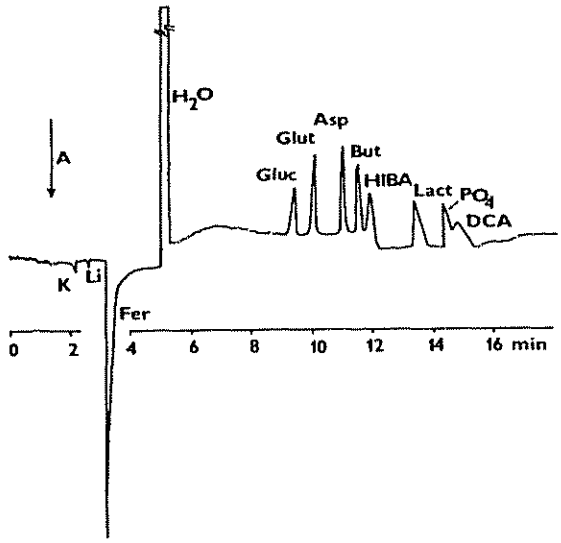


Fig. 8. Variation of migration times due to changes in electroosmotic flow caused by the sorption of the sample component. Fer = Ferroin. Other conditions as in Fig. 7.

to which 10^{-6} *M* ferroin was added. The sorption of ferroin on the capillary wall manifests itself not only by the tailing of the ferroin peak but also by a substantial decrease in the electroosmotic flow, which led to longer migration times and loss of the dimethylmalonate zone.

ACKNOWLEDGEMENTS

Our thanks are due to Mr. V. Monaco and Mr. R. Di Nino for the construction of the capillary holder for the Varian detector.

REFERENCES

1 P. Boček, M. Deml, P. Gebauer and V. Dolnik, *Analytical Isotachophoresis (Electrophoresis Library)*, Verlag Chemie, Weinheim, 1988.
2 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
3 P. Gebauer, M. Deml, P. Boček and J. Janák, *J. Chromatogr.*, 267 (1983) 455.
4 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 11.
5 F. Foret, M. Deml, V. Kahle and P. Boček, *Electrophoresis*, 7 (1986) 430.
6 X. Huang, M. J. Gordon and R. N. Zare, *J. Chromatogr.*, 425 (1988) 385.
7 Y. Walbrochl and J. W. Jorgenson, *Chromatographia*, 315 (1984) 135.
8 J. S. Green and J. W. Jorgenson, *J. Chromatogr.*, 352 (1986) 337.
9 R. A. Wallingford and A. G. Ewing, *Anal. Chem.*, 59 (1987) 1762.
10 J. A. Olivares, N. T. Nguyen, C. R. Yonker and R. D. Smith, *Anal. Chem.*, 59 (1987) 1230.
11 H. Small and T. E. Miller, Jr., *Anal. Chem.*, 54 (1982) 462.
12 M. Janeček and K. Šlais, *J. Chromatogr.*, 471 (1989) 303.
13 L. Arlinger and H. Lundin, *Protides Biol. Fluids, Proc. Colloq.*, 21 (1973) 667.
14 W. G. Kuhr and E. S. Yeung, *Anal. Chem.*, 60 (1988) 1832.
15 W. G. Kuhr and E. S. Yeung, *Anal. Chem.*, 60 (1988) 2642.
16 H. H. Lauer and D. McManigil, *Trends Anal. Chem.*, 1 (1986) 11.
17 F. Foret, M. Deml and P. Boček, *J. Chromatogr.*, 452 (1988) 601.
18 J. W. Jorgenson and K. D. Lukacs, *Science*, 222 (1983) 266.
19 H. H. Lauer and D. McManigil, *Anal. Chem.*, 58 (1986) 166.
20 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 1.
21 S. Hjertén, *Chromatogr. Rev.*, 9 (1967) 122.
22 T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi and J.-I. Akiyama, *J. Chromatogr.*, 271 (1983) D1.
23 K. D. Lukacs and J. W. Jorgenson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 407.
24 F. Foret and P. Boček, *Adv. Electrophoresis*, in press.

34016
Separation of Small Anions using Dichromate for Indirect UV Detection

A method of separation for anions with high mobilities has been developed for capillary electrophoresis. An osmotic flow modifier, diethylenetriamine, is used to suppress the electroosmotic flow. The sample is injected at the negative electrode so that the anions migrate before the neutral marker peak (water), leading to very short separation times.

Fresh buffer is prepared daily from 3 stock solutions. One solution of 180 mM potassium dichromate and 1.3 mM H_2SO_4 is diluted 1:100 into the running buffer for a final dichromate concentration of 1.8 mM. The second solution is 2.4 M borate (1.70 M $NaBO_2$ and 0.703 M $Na_2B_4O_7 \cdot 10H_2O$) combined with 50 mM diethylenetriamine. The second solution is diluted 1:50 into the running buffer for a final borate concentration of 48 mM and a final diethylenetriamine concentration of 1 mM, at pH 8.0. It is important to retain the oxidation state of the dichromate and for reagents to have a shelf life of at least several months. Thus, the diethylenetriamine and dichromate solutions are not combined until the day of use. Running conditions are -30 kV at 40°C in a 75 micron, 70 cm capillary. Samples are dissolved in water and electrokinetically injected for 5 sec.

Dichromate's high mobility in an electric field approximates the mobilities of the anions in this study. Dichromate is also suitable as a background electrolyte because it strongly absorbs ultraviolet light. Three of the anions separated, chloride, sulfate and oxalate, show negative peaks where these anions replace dichromate in solution because they absorb much less UV light than the dichromate ions which they displace. However nitrate and nitrate absorb strongly at 210 nm but not at 254 nm, so a positive peak is observed at the lower wavelength but not at the higher (see figure 1).

This technique represents a significant improvement over previous anion separation methods (W. R. Jones and P. Jandik, J. Chromatog. 546 (1991) 445-458) because here, nitrogen-containing anions are distinguished from other anions when absorption is simultaneously monitored at both 210 and 254 nm. For nitrate and nitrite, the limits of detection are lower at the shorter wavelength (50 ppb at 210 nm). For chloride and sulfate, the wavelength of choice is 254 nm (50 ppb). An improper choice of wavelength can lead to a decrease of sensitivity by 5-10 fold.

The selectivity of the separation is controlled by the temperature of the separation. Figure 1 shows the separation of five anions, chloride, nitrite, sulfate, nitrate, and oxalate, at 40°C. At 30°C (Figure 2), nitrate eluted before sulfate, and separation time is increased for both. At 30°C (not shown) nitrate and sulfate co-elute, illustrating the crucial role of temperature in selectivity of the separation. As temperature is increased, nitrate migration continues increase relative to chloride, so that resolution, optimal at 40°C, again degrades at higher temperatures. Control of the temperature is a significant contribution to the reproducibility and selectivity of this separation.

We have determined that the concentration of borate at 48 mM is optimal, because lower borate concentrations produce broader peaks and hence decreased resolution, but that 48 mM borate produces a current of less than 40 microamps in a 75 micron, 70 cm capillary run at -30 kV. The low current of this cocktail allows enhanced sensitivity by permitting the use of wider bore capillaries.

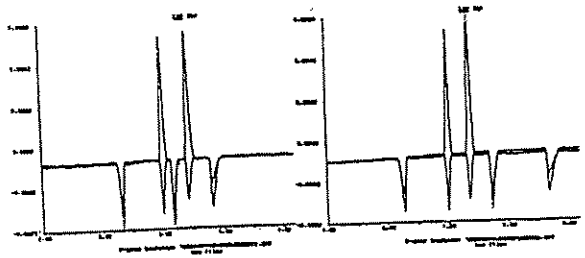


Figure 1 Separation of Cl^- NO_2^- $SO_4^=$ NO_3^- $C_2O_4^=$ (10 ppm each) at 40°C

Figure 2 Separation of Cl^- NO_2^- NO_3^- $SO_4^=$ $C_2O_4^=$ (10 ppm each) at 30°C

Disclosed by Spectra-Physics Analytical, Lenore Kelly and Dean S. Burgi.
34016

APPLICANT'S DISCLOSURE

Research Disclosure • August 1992 / 663

340106
Separation of Organic Acids using Phthalate Ion for Indirect UV Detection

INTRODUCTION

Capillary Zone Electrophoresis is a powerful method to separate small analytes. Many analytes, including most inorganic ions, do not absorb UV light, and thus pass the detector area without being observed. The use of light-absorbing ions in the separating buffer allows detection of ions that do not themselves contain a chromophore. The method of vacancy chromatography is illustrated here through the use of a phthalate-tetradecyltrimethylammonium bromide buffer to separate anions. Sample ions replace ions in the buffer as the sample band migrates through the capillary, so the charge density and ionic strength remain uniform throughout the solution in the capillary. More light passes through the detector region when a colorless ion locally reduces the concentration of a light-absorbing buffer component, and a negative peak is observed.

A number of applications for separation of low molecular weight anions with various carrier chromophore-containing electrolytes have been summarized (P. Jandik and W. R. Jones, J. Chromatogr. 546 (1991) 431). To lower detection limits below 1 ppm and to resolve some organic ions, method parameters still need to be improved. Electrokinetic injection can increase sample load and sensitivity compared to hydrodynamic introduction. Because mis-matched ionic mobilities of the carrier electrolytes and the sample ions can cause peak fronting or tailing, the choice of buffer components must be carefully considered. Additionally, the relative concentrations of ions to be separated to both that of the carrier electrolyte and of the light-absorbing buffer components determine the linear dynamic range of detectability. It has been previously shown that in similar systems, pH and the choice and concentration of electroosmotic flow modifiers had significant influence upon selectivity (W. R. Jones and P. Jandik, J. Chromatogr. 546 (1991) 445).

This paper describes the use of phthalate ion as a carrier electrolyte to separate the anions oxalate, tartrate, malate, succinate, lactate, acetate, propionate, butyrate and caprylate. In the presence of tetradecyltrimethylammonium bromide to reverse the electroosmotic flow, good resolution and peak shape of these anions is obtained. Easy to follow directions are given for preparation of stable concentrated buffer components, and a standard ion test mixture.

TO PREPARE BUFFER

A 50x concentrate of buffer is conveniently prepared by adding 10.2 g potassium acid phthalate, 1.68 g tetradecyltrimethylammonium bromide, and water to about 150 mL. After dissolving, the solution is adjusted to pH 6.0 with 1 N KOH, then diluted to 200 mL final volume. The working buffer is prepared from concentrate is diluted 2 mL plus 98 mL high-quality deionized water, and filtered with a 6 micron filter.

TO PREPARE ORGANIC ION STANDARD

Solutions of 10,000 ppm organic acid ion standards can be prepared by weighing any or all of the below, and dissolving individually in D.I. water to 10.0 mL solution.

141 mg oxalic acid
101 mg succinic acid
101 mg tartaric acid
128 mg sodium butyrate
131 mg sodium propionate
101 mg malic acid
134 mg sodium acetate
118 mg sodium caprylate
100 mL lactic acid solution

TEMPERATURE LOWER THAN RECITED IN CLAIM

Mixtures are then prepared by combining and diluting with D.I. water to 10 ppm, or as desired, and filtering.

EXPERIMENTAL CONDITIONS

Solutions of the organic anions in water are run at reverse polarity using a 5.0 mM potassium acid phthalate and 0.5 mM tetradecyltrimethylammonium bromide solution. Electrokinetic injection is at -10 kV. Under running conditions at 20°C at -30 kV on a fused silica 75 micron x 70 cm capillary, the current is about 20 microamps. UV detection is at 210 nm.

EXPERIMENTAL RESULTS

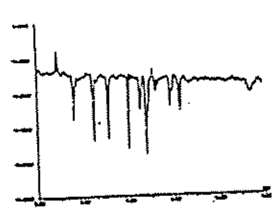


Figure 1. Organic acid standards after a 1 sec injection of a 10 ppm solution, run under conditions described in text. Elution order is oxalate, tartrate, malate, succinate, lactate, acetate, propionate, butyrate, caprylate.

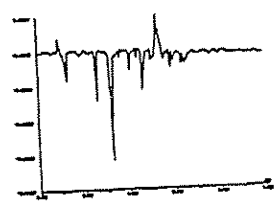


Figure 2. A 1:10 dilution of Chablis wine, electrokinetically injected for 1 sec and run under conditions described in text. Tartrate and malate are the major organic acids.

Disclosed by Spectra-Physics Analytical, Lenore Kelly
340106

Research Disclosure 8/10/93

Separation of Organic Acids using Phthalate Ion for Indirect UV Detection

INTRODUCTION

Capillary Zone Electrophoresis is a powerful method to separate small analytes. Many analytes, including most inorganic ions, do not absorb UV light, and thus pass the detector area without being observed. The use of light-absorbing ions in the separating buffer allows detection of ions that do not themselves contain a chromaphore. The method of vacancy chromatography is illustrated here through the use of a phthalate-tetradecyltrimethylammonium bromide buffer to separate anions. Sample ions replace ions in the buffer as the sample band migrates through the capillary, so the charge density and ionic strength remain uniform throughout the solution in the capillary. More light passes through the detector region when a colorless ion locally reduces the concentration of a light-absorbing buffer component, and a negative peak is observed.

A number of applications for separation of low molecular weight anions with various carrier chromophore-containing electrolytes have been summarized (P. Jandik and W. R. Jones, J. Chromatogr. 546 (1991) 431). To lower detection limits below 1 ppm and to resolve some organic ions, method parameters still need to be improved. Electrokinetic injection can increase sample load and sensitivity compared to hydrodynamic introduction. Because mis-matched ionic mobilities of the carrier electrolytes and the sample ions can cause peak fronting or tailing, the choice of buffer components must be carefully considered. Additionally, the relative concentrations of ions to be separated to both that of the carrier electrolyte and of the light-absorbing buffer components determine the linear dynamic range of detectability. It has been previously shown that in similar systems, pH and the choice and concentration of electroosmotic flow modifiers had significant influence upon selectivity (W. R. Jones and P. Jandik, J. Chromatogr. 546 (1991) 445).

This paper describes the use of phthalate ion as a carrier electrolyte to separate the anions oxalate, tartrate, malate, succinate, lactate, acetate, propionate, butyrate and caprylate. In the presence of tetradecyltrimethylammonium bromide to reverse the electroosmotic flow, good resolution and peak shape of these anions is obtained. Easy to follow directions are given for preparation of stable concentrated buffer components, and a standard ion test mixture.

TO PREPARE BUFFER

A 50x concentrate of buffer is conveniently prepared by adding 10.2 g potassium acid phthalate, 1.83 g tetradecyltrimethylammonium bromide, and water to about 150 mL. After dissolving, the solution is adjusted to pH 5.0 with 1 N KOH, then diluted to 200 mL final volume. The working buffer is prepared from concentrate is diluted 2 mL plus 98 mL high-quality deionized water, and filtered with a 5 micron filter.

TO PREPARE ORGANIC ION STANDARD

Solutions of 10,000 ppm organic acid ion standards can be prepared by weighing any or all of the below, and dissolving individually in D.I. water to 10.0 mL solution.

141 mg oxalic acid
101 mg succinic acid
101 mg tartaric acid
126 mg sodium butyrate
131 mg sodium propionate
101 mg malic acid
138 mg sodium acetate
116 mg sodium caprylate
100 mL lactic acid solution

Mixtures are then prepared by combining and diluting with D.I. water to 10 ppm, or as desired, and filtering.

EXPERIMENTAL CONDITIONS

Solutions of the organic anions in water are run at reverse polarity using a 5.0 mM potassium acid phthalate and 0.5 mM tetradecyltrimethylammonium bromide solution. Electrokinetic injection is at -10 kV. Under running conditions at 20°C at -30 kV on a fused silica 75 micron x 70 cm capillary, the current is about 20 microamps. UV detection is at 210 nm.

EXPERIMENTAL RESULTS

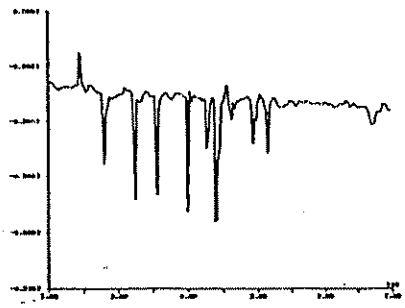


Figure 1. Organic acid standards after a 1 sec injection of a 10 ppm solution, run under conditions described in text. Elution order is oxalate, tartrate, malate, succinate, lactate, acetate, propionate, butyrate, caprylate.

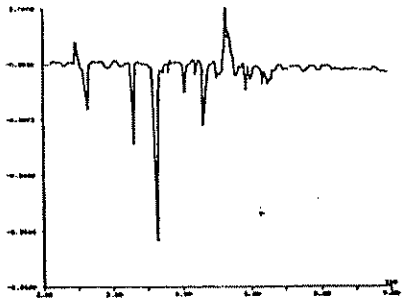


Figure 2. A 1:10 dilution of Chablis wine, electrokinetically injected for 1 sec and run under conditions described in text. Tartrate and malate are the major organic acids.

Disclosed by Spectra-Physics Analytical, Lenore Kelly

J. Chrom. 1993
Vol. 16 No. 9 10 pp. 2103-2122

CAPILLARY ZONE ELECTROPHORESIS OF ORGANIC ACIDS AND ANIONS

L. KELLY AND R. J. NELSON

SPECTRA-PHYSICS ANALYTICAL, INC.

45757 NORTHPORT LOOP WEST

P.O. BOX 5116

FREMONT CA 94537-5116

ABSTRACT

Two buffer systems are described for the separation of anions by capillary electrophoresis. In each system, the ions are monitored by indirect detection. Organic acids are separated with potassium hydrogen phthalate, 2-(N-morpholino)-ethanesulfonic acid and tetradecyltrimethyl ammonium bromide. Other anions are separated in 1,2,4,5 benzene tetracarboxylic acid (pyromellitic acid) and diethylenetriamine.

INTRODUCTION

Capillary Zone Electrophoresis is a powerful method to separate small analytes (1-4). Most inorganic ions do not absorb UV light and pass the detector area without a change in signal. The use of light-absorbing ions as buffer components allows indirect detection of ions that do not themselves contain a chromophore (5,6). This report describes indirect anion detection with two buffer systems.

For indirect detection, it is the absorbance of a buffer electrolyte that is monitored by the detector, not the absorptivity that the sample might display. Because the solution in the capillary is constrained to remain electrically neutral, sample ions locally displace electrolyte on a charge-for-charge basis as the sample band migrates through the capillary. As the buffer electrolyte is diluted by the sample ions, more photons pass through the detector region. The increase in light throughput is recorded as a decrease in absorbance. The magnitude of the negative peak is dependent upon the concentration of the displacing ion, the ratio of the negative charges on the electrolyte to the sample ion, and finally, the concentration and extinction coefficient of the electrolyte.

A number of applications for separation of low molecular weight anions with various chromophore-containing electrolytes have been summarized (7). To lower detection limits below 1 ppm and to resolve some co-migrating inorganic ions, method parameters are still being improved. Electrokinetic injection can increase sample loading and sensitivity compared to hydrodynamic introduction although a bias does exist (8). Because mis-matched ionic mobilities of the carrier electrolytes and the sample ions produce peak fronting or tailing, choice of buffer components must be carefully considered (5,9). It has been previously shown that in similar systems, pH as well as the choice and concentration of electroosmotic flow modifiers had significant influence upon selectivity (10). The influence of yet another factor, the choice of running temperature upon the selectivity of separation, is further investigated below.

Here, the use of phthalate for the separation and detection of organic acids, and of 1,2,4,5 benzene tetracarboxylic acid for anions is shown. The critical role of temperature in selectivity of the anion separation is also discussed.

MATERIALS

Capillary electrophoresis was conducted with the SpectraPHORESIS™ 1000 (Spectra-Physics Analytical, Fremont, CA). Capillaries were untreated fused silica, 70 cm x 75 µm or 44 cm x 50 µm.

All chemicals were obtained at the highest purity level available from the manufacturer, and were used without additional purification. n-Butyric acid (sodium salt), caprylic acid, D,L-malic acid, 2-(N-morpholino)-ethanesulfonic acid (MES), oxalic acid, potassium hydrogen phthalate, propionic acid (sodium salt), succinic acid, L-tartaric acid, and tetradecyltrimethyl ammonium bromide (TTAB) were obtained from Sigma Chemical Co., St. Louis MO. 1,2,4,5 benzenetetracarboxylic acid (pyromellitic acid or PMA), and diethylenetriamine (DETA), were obtained from Aldrich Chemical Co., Milwaukee, WI. HPLC grade water from Baker or 18 MΩ Milli-Q water was used.

PREPARATION OF BUFFERS

For organic acid separations, a 10x buffer concentrate was made from 50 mM potassium acid phthalate, 5 mM TTAB, and 500 mM MES adjusted to pH 5.2 with NaOH (Spectra-Physics Part

Number A4452-010). When diluted 1:10, the running buffer was 5 mM potassium acid phthalate, 0.5 mM TTAB, and 50 mM MES. Sample injection was electrokinetic for 1 sec at -10 kV. Capillary electrophoresis was at 20° C and -30 kV, and the current was less than 20 µA using a 70 cm x 75 µm capillary. Detection was at 205 nm.

For anion separations, the running buffer was 3 mM pyromellitic acid, 0.02% DETA, 1% methanol at pH 9.6. To avoid solubility problems, the PMA was dissolved in 1 mL of methanol, added to water containing 7mM NaOH, and combined with DETA. The pH was then adjusted further with NaOH as required. Sample injection was electrokinetic for 5 sec at -10 kV. Capillary electrophoresis was at 60° C and -25 kV, and the current was less than 40 µA using a 44 cm x 50 µm capillary. Separate capillaries were maintained for each of the buffer formulations.

RESULTS AND DISCUSSION

Separation of a series of organic acids is shown in Figure 1. TTAB has been added to the phthalate buffer to reverse the electroosmotic flow, so the polarity has been reversed for this series of separations. The inclusion of the Good's buffer, MES into the phthalate mixture provided stabilization against pH changes as well as an improved baseline and better peak shape

compared to the same mixture without MES (data not shown). A field-amplified concentration (11) of the sample bands shown in Figure 1 results from the electrokinetic injection of sample diluted in water into the capillary containing relatively concentrated electrolyte. The presence of organic acids can be detected in beverages such as white wine and brewed coffee, as shown in Figures 2 and 3.

Smaller anions show better peak shapes when the mobility of absorbing buffer ion more closely matches that of the displacing sample ions. PMA, with two more carboxylate groups than phthalate, has a higher mobility due to its higher charge density. Mobility of the PMA is enhanced at pH 9.6, where the acidic carboxylates are predominately ionized. In the PMA system, DETA is used to slow rather than reverse the electroosmotic flow. Of the anions shown in Figure 4, peak shape is broader for the larger anions, e.g., bromate.

Figure 5 shows the effects of temperature upon the migration times of chloride, nitrite, sulfate, nitrate and oxalate. As expected, increasing the temperature causes solution viscosity to decrease, so migration times are also decreased. However, the selectivity of the system is also observed to change with temperature. Nitrite and nitrate migrate with the same relationship to each other, while chloride, sulfate and oxalate also maintain their same relative distances. Because the nitrogen-containing ions migrate relatively slower than the other three as

the temperature is increased, the elution order is a marked function of temperature. The optimal temperature for this separation is 60°C. The buffer mixture was adjusted to pH 9.6 at 23°C, and was used unadjusted for the temperature studies since temperature does not appreciably affect the ionization of carboxylates.

CONCLUSIONS

Small anions are readily separated by CE. Indirect detection is used to detect these ions, most of which do not absorb ultraviolet light. Choice of the indirect chromaphore is dictated by the mobility of the ions to be analyzed, since the best resolution occurs when the mobility of the anionic buffer is close to that of the sample ions. The speed of the analysis and in some cases the selectivity are controlled by the temperature.

REFERENCES

1. J.W. Jorgensen. and K.D. Lukacs, Anal. Chem., 53: 1298-1302 (1981)

2. F.E.P. Mikkers, F.M. Everarts, and Th.P.E.M. Verheggen, J. Chromatogr., 169: 11-20 (1979)

3. X.H. Huang, J.A. Luckey, M.J. Gordon, and R.N. Zare, Anal. Chem., 61, 766-770 (1989)

4. W.G. Kuhr, and E.S. Yeung, Anal. Chem., 60: 2642-2640 (1988)

5. F. Foret, S. Fanali, A. Nardi, and P. Bocek, Electrophoresis, 11: 780-782 (1990)

6. W.D. Pfeffer, T. Takeuchi, and E.S. Yeung, Chromatographia, 24: 123-125 (1987)

7. P. Jandik, and W. R. Jones, J. Chromatogr., 546: 431-443 (1991)

8. X. Huang, M.J. Gordon, and R.N. Zare, Anal. Chem., 60: 375-377 (1988)

9. S. Hjerten, K. Elenbring, F. Kilar, J.-L. Liao, A.J.C. Chen, C.J. Siebert and M.-D. Zhu, J. Chromatogr., 403: 47-61 (1987)

10. W. R. Jones, and P. Jandik, J. Chromatogr., 546: 445-458 (1991)

11. R.-L. Chien, and D.S. Burgi, Anal. Chem., 64: 489A-496A (1992)

Figure Legends

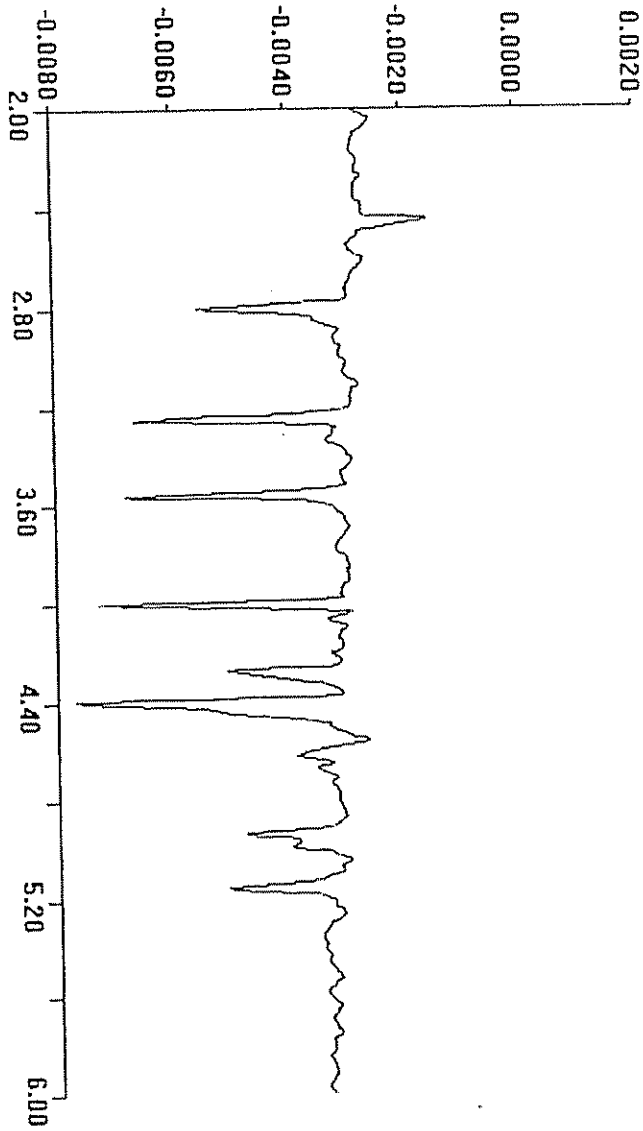
Figure 1 10 ppm Each of the organic ions oxalate, tartarate, malate, succinate, lactate, followed by the water peak at 4.6 min, then propionate. Caprylate can be seen as the broad peak at 6.8 min. Capillary electrophoresis was at 20° C and -30 kV in phthalate buffer.

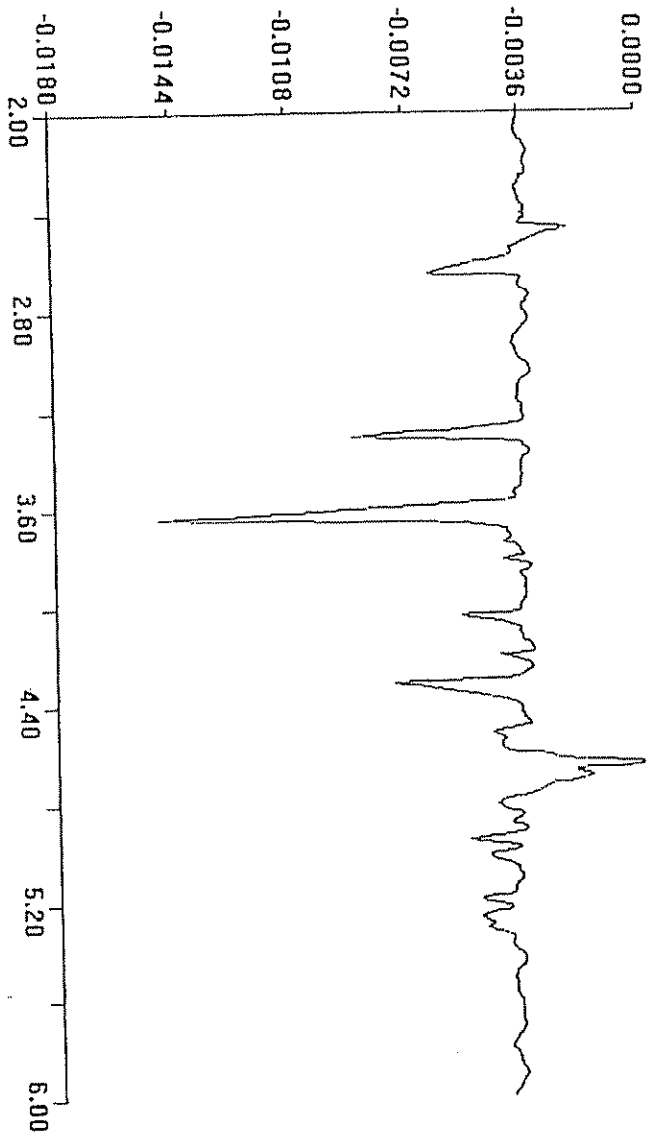
Figure 2 Tartarate and malate are the major organic acids in white wine. Conditions for the phthalate buffer are given in Figure 1.

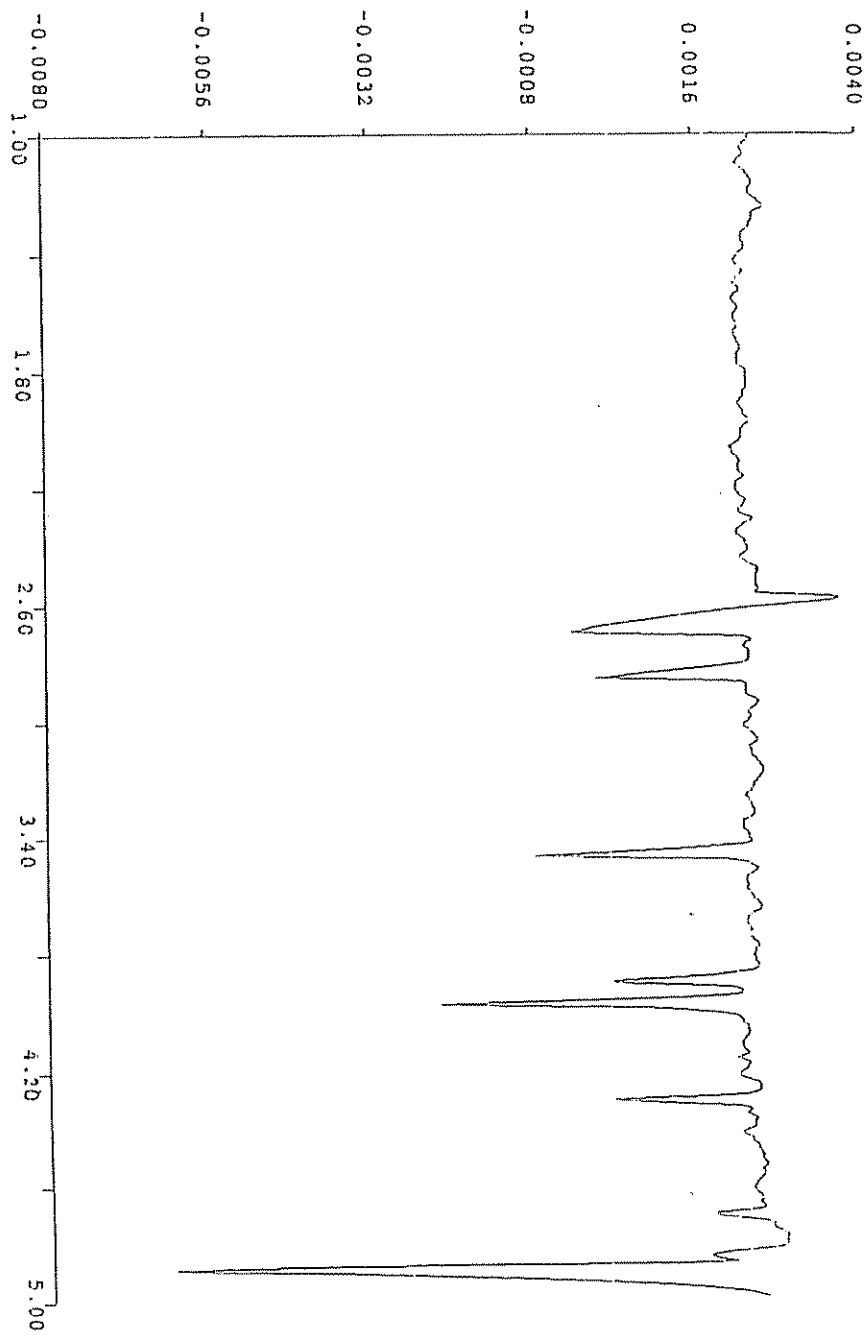
Figure 3 Organic acids in coffee, diluted 1:10 in water. Conditions for the phthalate buffer are given in Figure 1.

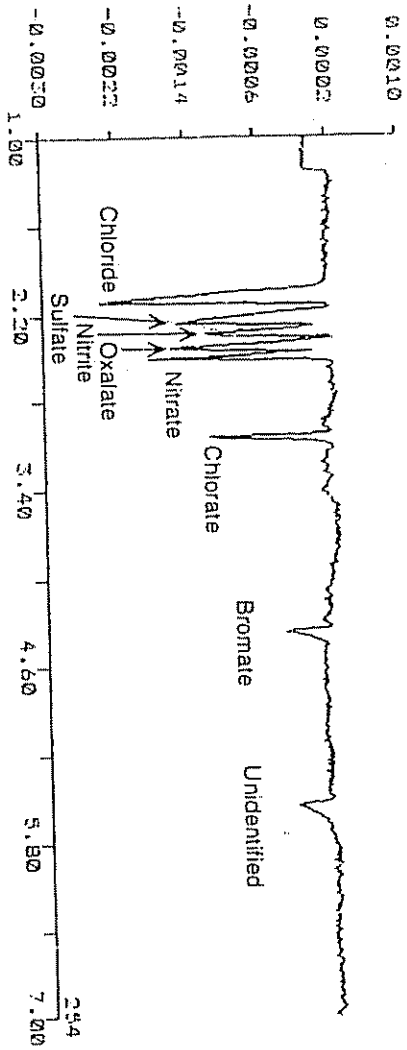
Figure 4 5 ppm Chloride, sulfate, nitrite, oxalate, nitrate, chlorate, and bromate have been separated using the PMA buffer. The broad peak at 5.5 min is unidentified. Following electrokinetic injection for 5 sec at -10 kV, capillary electrophoresis was at 60° C and -25 kV.

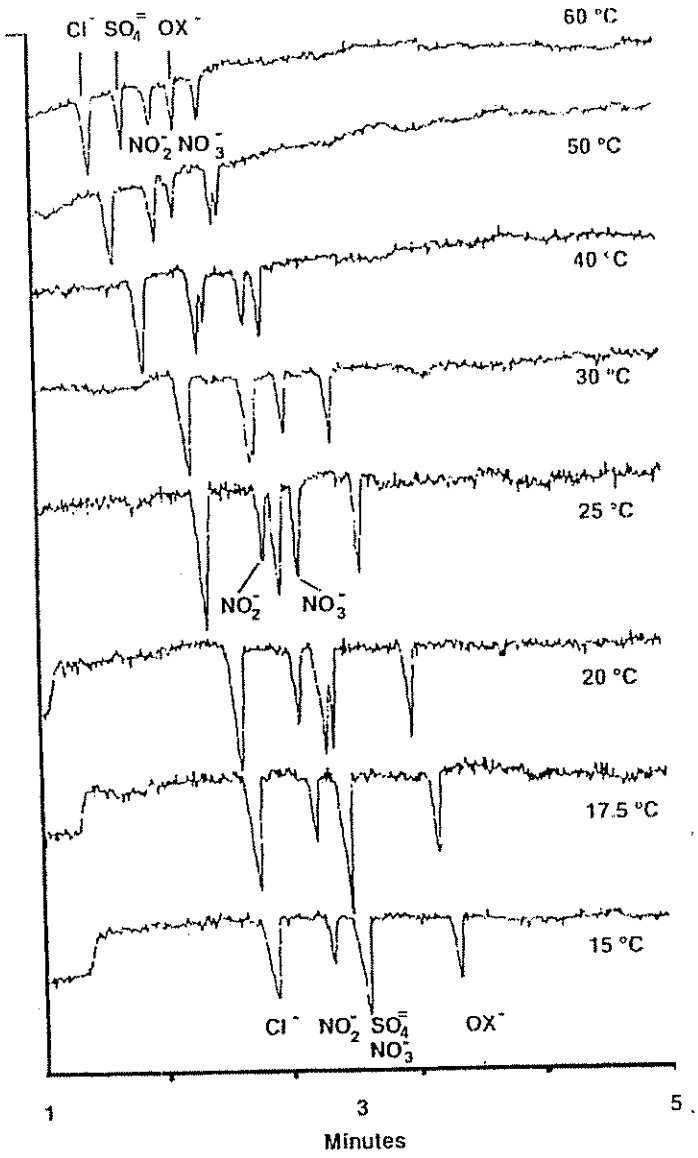
Figure 5 The effects of temperature variation upon migration times and selectivity of anions in the PMA buffer system are shown in the 15° - 60°C range. Chloride, sulfate and oxalate migrate relatively faster than the nitrite-nitrate pair as the temperature is increased. Baseline resolution of the 5 ions is only observed at 60°C. Conditions are as for Figure 4 at the indicated temperature, except that a 1 ppm solution is electrokinetically injected for 1 sec.


-0.0080
-0.0060
-0.0040
-0.0020
0.0000
0.0020
2.00
2.80
3.60
4.40
5.20
6.00


-0.0180
-0.0144
-0.0108
-0.0072
-0.0036
0.0000
2.00
2.80
3.60
4.40
5.20
6.00


-0.0080
-0.0056
-0.0032
-0.0008
0.0016
0.0040
1.00
1.80
2.60
3.40
4.20
5.00


0.0010
0.0002
-0.0006
-0.0014
-0.0022
-0.0030
1.00
2.20
3.40
4.60
5.80
7.00
254
Chloride
Sulfate
Nitrite
Oxalate
Nitrate
Chlorate
Bromate
Unidentified


Cl⁻ SO₄⁼ OX⁻
60 °C
NO₂⁻ NO₃⁻
50 °C
40 °C
30 °C
25 °C
NO₂⁻
NO₃⁻
20 °C
17.5 °C
15 °C
Cl⁻ NO₂⁻ SO₄⁼ NO₃⁻
OX⁻
1
3
5
Minutes

IIC-2/8/94
OAP 10/29/93
Stanicki

KILLWORTH, GOTTMAN, HAGAN & SCHAEFF
ATTORNEYS AT LAW
ONE DAYTON CENTRE
ONE SOUTH MAIN STREET, SUITE 500
DAYTON, OHIO 45402-2023
513/223-2050
FACSIMILE: 513/223-0724

RICHARD A. KILLWORTH
JAMES F. GOTTMAN
TIMOTHY W. HAGAN
B. JOSEPH SCHAEFF
RICHARD C. STEVENS
ROBERT I. SHOWALTER
KIMBERLY GAMBREL

SCOT R. HEWITT
BRIAN M. BOLAM
JOHN R. FLANAGAN*
CHARLES R. WILSON
OF COUNSEL
*Admitted in Connecticut only

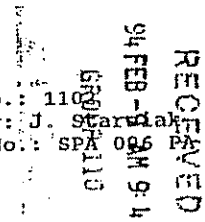
#4

Date: January 17, 1994

In re Application of

Inventor(s) : Lenore Kelly, Dean S. Burgi, Robert J. Nelson
Serial No. : 08/088,439
Filed : July 7, 1993
Title : CONTROLLED TEMPERATURE ANION SEPARATION BY CAPILLARY ELECTROPHORESIS

Group No.: 1103
Examiner: J. Stanicki
Docket No.: SPA 086 PA



Hon. Commissioner of Patents and Tradmarks
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

_____ No additional fee is required.
__X__ The fee has been calculated as shown below:

	Claims Remaining After Amendment		Highest Number Previously Paid For		Present Extra		Rate		Additional Fee
Total Claims	19	Minus	19	=	0	X	$22	=	$ 00.00
Indep. Claims	5	Minus	3	=	2	X	$74	=	$148.00
Surcharge for Multiple Dependent Claims Added by this Amendment ($230.00)									$ 00.00
Total Additional Fee for this Amendment									$148.00

Enclosed is our check in the amount of $148.00 which represents the payment of the above filing fee(s).

Respectfully submitted,

KILLWORTH, GOTTMAN, HAGAN & SCHAEFF

By _____
Timothy W. Hagan
Reg. No. 29,001

TWH/AMS

148@-182

GP1102, #4/Amdt. St. 2/25/94

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of

Applicants : Lenore Kelly, Dean S. Burgi, Robert J. Nelson
Serial No. : 08/088,439
Filed : July 7, 1993
Title : CONTROLLED TEMPERATURE ANION SEPARATION BY CAPILLARY ELECTROPHORESIS

B.H. 2-25-94

Docket : SPA 096 PA
Examiner : J. Starsiak
Art Unit : 1102

Hon. Commissioner of Patents and Trademarks
Washington, D C. 20231

Sir:

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D C. 20231, on January 17, 1994.

Timothy W. Hogan 29,007
Attorney Reg. No.

RECEIVED 94 FEB -9 AM 9:43 GROUP 1102

AMENDMENT

This paper is being filed in response to the Office Action mailed October 20, 1993. Reconsideration and reexamination are respectfully requested in light of the amendments and remarks below.

IN THE CLAIMS

Please amend claim 1 to read as follows:

1. (Amended) A method for detecting and separating anions in a sample using capillary electrophoresis comprising the steps of, providing a capillary filled with a carrier electrolyte, said carrier electrolyte containing a light-absorbing co-anion, heating or cooling said capillary to a target temperature [different from ambient temperature] in the range of from 25°C to 60°C, introducing a sample containing one or more anions into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, and

090 BA 02/07/94 08088439 1 102 148.00 CK

A

A1 Cont.

detecting said anions indirectly using a photometric detector while maintaining the temperature in said capillary to within ±0.5°C of said target temperature.

Please cancel claims 2 and 3.

Please amend claim 4 at line 2 by changing "40.°C" to --40°C--.

Please rewrite claim 5 in independent form to read as follows:

A2 5. (Amended) [The] A method [of claim 1] for detecting and separating anions in a sample using capillary electrophoresis comprising the steps of, providing a capillary filled with a carrier electrolyte, heating or cooling said capillary to a target temperature in the range of from 25°C to 60°C, introducing a sample containing one or more anions into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, [in which said] varying said target temperature [is varied] as said anions migrate, and detecting said anions.

Please amend claim 6 at line 1 by changing "2" to --1--.

Please amend claim 7 at line 1 by changing "2" to --1--.

Please rewrite claim 10 in independent form to read as follows:

A3 10. (Amended) [The] A method [of claim 9] for detecting and separating anions in a sample using capillary electrophoresis comprising the steps of, providing a capillary filled with a carrier electrolyte and diethylenetriamine as an electroosmotic flow modifier, [in which said electroosmotic flow modifier is diethylenetriamine] heating or cooling said capillary to a target temperature in the range of from 25°C

a3 end

to 60°C, introducing a sample containing one or more anions into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, and detecting said anions.

Please amend claim 12 at line 1 by changing "2" to --1-- and at line 3, by deleting "acetate,".

Please amend claim 14 to read as follows:

11 14. (Amended) A method for detecting and separating anions in a sample using capillary electrophoresis comprising the steps of, providing a capillary filled with a carrier electrolyte, heating or cooling said capillary to a target temperature in the range of from 20° to 60°C, introducing a sample containing one or more anions [selected from the group consisting of chloride, nitrate, nitrite, sulfate, and oxalate anions] into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, and detecting said anions by simultaneously monitoring said sample at two different wavelengths while maintaining the temperature in said capillary to within ±0.5°C of said target temperature.

14

Please rewrite claim 19 in independent form to read as follows:

17 18. (Amended) [The] A method [of claim 14 including the step of] for detecting and separating anions in a sample using capillary electrophoresis comprising the steps of, providing a capillary filled with a carrier electrolyte, heating said capillary to a target temperature in the range of from 20° to 60°C, introducing a first portion of a sample containing one or more anions into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, detecting said anions by

15

simultaneously monitoring said sample at two different wavelengths, introducing a second portion of said sample into said capillary containing one or more anions, changing said temperature in said capillary, detecting said anions in said second portion, and comparing the order of elution of said anions in said second portion with said first portion.

Please add new claims 20 and 21 to read as follows:

20. The method of claim 1 in which said light absorbing co-anion is selected from the group consisting of pyromellitate, chromate, and dichromate.

21. The method of claim 15 in which said light absorbing co-anion is selected from the group consisting of pyromellitate, chromate, and dichromate.

REMARKS

Applicants' invention is directed to a method for the separation of anions using capillary electrophoresis techniques. Both organic and inorganic anions may be separated. Using precise control of the temperature of the fluid in the capillary column, the migration speed and order of migration of the anions may be controlled to improve the selectivity of the process. Because the viscosity of the electrolyte solution in which the sample ions migrate is influenced by temperature, close temperature control provides a high degree of reproducibility for samples and enables one to track and identify specific anions. Further, by proper temperature selection, the order of elution of anions may be controlled as well as the selectivity of their separation.

Applicants note the Examiner's indication of allowable subject matter in dependent claims 5, 8, 10, 16, 18, and 19. Several of those claims have now been rewritten in

independent form to place them in condition for allowance. Thus, claim 5 has been rewritten to overcome the §112 rejection and recites varying the target temperature as the anions migrate. Claim 10 has been rewritten to overcome the §112 rejection and recites the use of diethylenetriamine as an electroosmotic flow modifier. Claim 19 has also been rewritten and recites the introduction of a second sample portion, changing the capillary temperature, and comparing the order of elutions of the first and second sample portions.

In the first Office Action, the Examiner rejected claims 1-12 under 35 USC §112, second paragraph, as being indefinite. Specifically, the language of claim 1 reciting a target temperature different than ambient temperature was deemed indefinite because ambient temperature is not a "fixed" temperature. Applicants have amended claim 1 by deleting the reference to ambient temperature and instead reciting the temperature range previously found in claim 3 for the target temperature. Claim 1 as amended now recites a specific range of temperatures and is believed to overcome the indefiniteness problem.

Also in the first Office Action, the Examiner rejected claims 1, 3, and 4 under 35 USC §102 as anticipated by Morin. However, claim 1 has now been amended to include the recitation found in original claim 2 that the carrier electrolyte contains a light-absorbing co-anion and that anions are detected indirectly using a photometric detector. Accordingly, as Morin does not teach or suggest such a method, applicants submit that the rejection has been overcome.

Also in the first Office Action, the Examiner rejected claims 1, 2, 6, 7, 11-15, and 17 under 35 USC §102 as anticipated by Jones et al, or alternatively under 35 USC §103 as unpatentable over Jones et al in view of Morin.

Jones et al, U.S. Patent No. 5,104,506, was cited by applicants in the Background section of the application and describes the use of indirect photometric techniques for the separation and detection of samples containing mixtures of common ionic species.

With respect to the rejection under §102, applicants have now amended claim 1 to recite the specific target temperature range found in original claim 3. As Jones et al are silent concerning any heating or cooling of the capillary, the reference does not teach or suggest a method of analysis which uses heating or cooling of a capillary to the presently recited range of temperatures. Accordingly, applicants submit that the §102 rejection has been overcome.

Further, with respect to the §103 rejection, the Examiner asserted that Morin shows that temperature can affect the "quality of the separation of anions using capillary zone electrophoresis" and thus it would have been obvious to "optimize the temperature used when performing the method of Jones et al because the criticality of this parameter is known in the art". However, Morin does not relate to an indirect absorbance detection system. Further, what Morin concluded was that selectivity for the particular arsenic ion separation was improved by adjusting pH (see Summary on page 357). With respect to temperature, Morin concluded that "temperature variations in the range 25-40°C did not significantly alter the selectivity of the separation at pH 5.6" (page 362). One skilled in the art would not conclude from Morin that temperature was a "critical parameter" as alleged by the Examiner. Moreover, there is no suggestion in either of the references that temperature affects separation selectivity or elution order.

With respect to Jones et al, the Examiner pointed to a passage at col. 5, lines 62-64, that detection was carried out at two different wavelengths, 254 and 272 nm. Applicants

presume that the Examiner was relying on this passage to reject claims 7 and 14 which recite detecting the anions by simultaneously monitoring the sample at two different wavelengths. However, Jones et al do not teach simultaneous monitoring at two wavelengths. Compare Jones et al at col. 4, lines 34-36 where it is stated that the anions of Jones et al were detected by monitoring the absorbance of the carrier electrolyte at 254 or 272 nm. None of the graph figures show simultaneous detection data, and the Example 3 detection was carried out at a single wavelength. As Jones et al do not teach simultaneous detection, claims 7 and 14 are patentable for these additional reasons.

With this response, applicants are also filing the attached Declaration by the inventors explaining that the disclosure contained in certain published articles cited to the Examiner in an Information Disclosure Statement mailed October 11, 1993 represented their joint work and do not constitute prior art with respect to the claimed invention.

For all of the above reasons, applicants submit that claims 1 and 4-19, as amended, and new claims 20-21 are patentable over the applied art of record and in compliance with §112. Early notification of the allowance of all remaining claims is respectfully solicited.

Respectfully submitted,
KILLWORTH, GOTTMAN, HAGAN & SCHAEFF

By _____
Timothy W. Hagan
Registration No. 39,001

One Dayton Centre
One South Main Street, Suite 500
Dayton, Ohio 45402-2023
(513) 223-2050
Facsimile: (513) 223-0724

#5/Declara.
Jt. 2/25/94

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of

Applicant : Lenore Kelly, Dean S. Burgi, Robert J. Nelson
Serial No. : 08/088,439
Filing Date : July 7, 1993
Title : CONTROLLED TEMPERATURE ANION SEPARATION BY CAPILLARY ELECTROPHORESIS
Docket : SPA 096 PA
Art Unit : 1102

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

DECLARATION

We, Lenore Kelly, Dean S. Burgi, and Robert J. Nelson, declare and state as follows:

We are co-inventors of the subject matter described and claimed in the United States Patent Application Serial No. 08/088,439, filed July 7, 1993, entitled CONTROLLED TEMPERATURE ANION SEPARATION BY CAPILLARY ELECTROPHORESIS (the "'439 application").

The following articles have been cited in an Information Disclosure Statement in the above-referenced patent application:

1. L. Kelly and R.J. Nelson, "Capillary Zone Electrophoresis of Organic Acids and Anions," J. Liq. Chrom., Vol. 16, Nos. 9 and 10, pp. 2103-2122, 1993.

2. L. Kelly, "Separation of Organic Acids Using Phthalate Ion for Indirect UV Detection," Research Disclosure, August 1993.

3. L. Kelly and D. Burgi, "Separation of Small Anions Using Dichromate for Indirect UV Detection," Research Disclosure, August 1992.

All of the above articles were published less than one year prior to the filing date of our '439 patent application. While we did not jointly coauthor each of the publications listed above, those articles describe our joint work on the invention described and claimed in our '439 patent application. Thus, all

of the authors listed contributed to the subject matter which we jointly disclose and claim in our '439 patent application.

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Lenore Kelly

Dean S. Burgi

Robert J. Nelson

112

J. D.
3/8/94
#6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:

BURGI DS FEB 28 PM 8:01

Applicant : KELLY L
NELSON RJ
Serial No.: 088439
Filed : 07/07/93
Docket No.: SPA096
Art Unit : 1102
Examiner : Starsiak, J.

Hon. Commissioner of Patents
and Trademarks
Washington DC 20231

Sir:

NOTICE OF CHANGE OF ADDRESS

Please be advised that the undersigned attorneys of record, having a full Power of Attorney presently on file in this U.S. patent application, have changed their address. The Patent and Trademark Office is requested to address all future correspondence to:

Killworth, Gottman, Hagan & Schaeff
One Dayton Centre
One South Main Street, Suite 500
Dayton OH 45402-2023

The telephone number and facsimile number have not been changed.

Respectfully submitted,

KILLWORTH, GOTTMAN, HAGAN
& SCHAEFF

By Richard A. Killworth
Richard A. Killworth
Registration No. 26,397

One Dayton Centre
One South Main Street, Suite 500
Dayton OH 45402-2023
(513)223-2050

Dated: 2/14/94

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/088,439	07/07/93	KELLY L	SPA096PA

STARSIAK, EXAMINER

D1M1/0506

KILLWORTH, GOTTMAN, HAGAN & SCHAEFF
ONE DAYTON CENTRE
ONE SOUTH MAIN STREET, SUITE 500
DAYTON, OHIO 45402-2023

ART UNIT	PAPER NUMBER
1102	7

DATE MAILED: 05/06/94

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 1/21/94 ☒ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), _____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☒ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 1 and 4-21 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☒ Claims 2 and 3 have been cancelled.
3. ☒ Claims 5, 10, and 19 are allowed.
4. ☒ Claims 1, 4, 6, 9, 11, 12, 14, 15, 17, 20, and 21 are rejected.
5. ☒ Claims 7, 8, 14, 16, and 18 are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings ☐ are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved. ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

PTOL-326 (Rev. 9-89)

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Evaluations of the level of ordinary skill in the art requires consideration of such factors as various prior art approaches, types of problems encountered in the art, rapidity with which innovations are made, sophistication of technology involved, educational background of those actively working in the field, commercial success, and failure of others.

The "person having ordinary skill" in this art has the capability of understanding the scientific and engineering principles applicable to the claimed invention. The evidence of record including the references and/or the admissions are considered to reasonably reflect this level of skill.

Claims 1, 4, 6, 9, 11, 12, 13, 15, 17, 20, and 21 are rejected under 35 U.S.C. § 103 as being unpatentable over Jones et al in view of Morin et al.

See first Office action for details.

Claims 7, 8, 14-16 and 18 are allowable over the prior art of record.

Applicant's arguments filed January 21, 1994 have been fully considered but they are not deemed to be persuasive.

Applicant's statement that "One skilled in the art would not conclude from Morin that the temperature was a "critical parameter" as alleged by the Examiner", is not well-taken because it ignores the following recitation in Morin et al, "At a temperature of 40°C, the peaks were sharper, the efficiency was slightly better and migration times were shorter". Also, Morin et al explicitly shows the relationship between electrophoretic mobility and temperature, see fig. 7.

Applicant's amendment necessitated the new grounds of rejection. Accordingly, THIS ACTION IS MADE FINAL. See M.P.E.P. § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. § 1.136(a).

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 C.F.R. § 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

Serial No. 088,439

Art Unit 1102

Any inquiry concerning this communication should be directed to John S. Starsiak, Jr. at telephone number (703) 308-0661.

J.Starsiak:mm
May 03, 1994

John Niebling
Supervisory Patent Examiner
Patent Examining Group 110

Corres. and Mail

BOX AF

Response Under 37 CFR §1.116
Expedited Procedure - Examining Group 1102

RECEIVED
JUL 0 8 1994
GROUP 1100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of

Applicant	:	Lenore Kelly, Dean S. Burgi, Robert J. Nelson
Serial No.	:	08/088,439
Filed	:	July 7, 1993
Title	:	CONTROLLED TEMPERATURE ANION SEPARATION BY CAPILLARY ELECTROPHORESIS
Docket	:	SPA 096 PA
Examiner	:	J. Starsiak
Art Unit	:	1102

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on July 1, 1994.

Attorney — Reg. No. 29,001

AMENDMENT AFTER FINAL REJECTION

This paper is being filed in response to the Office Action mailed May 6, 1994. Reconsideration and reexamination are respectfully requested in light of the amendments and remarks below. Entry of this amendment is respectfully requested as it is believed to place all of the remaining claims, 1, 4-6, and 8-21, in condition for allowance.

IN THE CLAIMS

Please amend claim 1 to read as follows:

1. (Twice amended) A method for detecting and separating anions in a sample using capillary electrophoresis comprising the steps of, providing a capillary filled with a carrier electrolyte, said carrier electrolyte containing a light-absorbing co-anion, heating or cooling said capillary to a target temperature in the range of from 25°C to 60°C, introducing a sample containing one or more anions into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, and detecting said anions indirectly using a photometric detector while maintaining the temperature in said capillary to within ±0.5°C of said target temperature, wherein said anions are detected by simultaneously monitoring said sample at two different wavelengths.

Please cancel claim 7.

Please amend claim 8, line 1, by changing "7" to --1--.

REMARKS

In the latest Office Action, the Examiner indicated that claims 5, 10 and 19 were allowed. Claims 7, 8, 14, 16 and 18 were objected to but indicated as being allowable over the prior art of record.

With the above amendment, the limitations of claim 7 have been incorporated into independent claim 1. With that amendment, applicants submit that claim 1 and claims 4, 6, 8-9, 11-13 and 20, which depend therefrom, are also in condition for allowance.

Applicants note that although independent claim 14 was indicated as being rejected on the face of the Office Action, it was indicated as being allowable in the body of the action. Accordingly, applicants believe that claim 14, and claims 15-18 and 21, which depend therefrom, are also in condition for allowance.

For all of the above reasons, applicants submit that claims 1, 4-6, and 8-21, all of the claims remaining in the application, are now in condition for allowance. Entry of this amendment and early notification of allowance is respectfully requested.

Respectfully submitted,
KILLWORTH, GOTTMAN, HAGAN
& SCHAEFF

By _____
Timothy W. Hagan
Registration No. 29,001

One Dayton Centre
One South Main Street, Suite 500
Dayton, Ohio 45402-2023
(513) 223-2050
Facsimile: (513)223-0724
/sml

B

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/088,439	07/07/93	KELLY	L SPA096PA

EXAMINER
STARSIAK, J

D1M1/0714

KILLWORTH, GOTTMAN, HAGAN & SCHAEFF
ONE DAYTON CENTRE
ONE SOUTH MAIN STREET, SUITE 500
DAYTON, OHIO 45402-2023

ART UNIT	PAPER NUMBER
1102	9

DATE MAILED: 07/14/94

NOTICE OF ALLOWABILITY

PART I.

1. ☒ This communication is responsive to applicant's communication filed 5 July 1994
2. ☒ All the claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice Of Allowance And Issue Fee Due or other appropriate communication will be sent in due course.
3. ☒ The allowed claims are 1,4-6, and 8-21
4. ☐ The drawings filed on _____ are acceptable.
5. ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has [_] been received. [_] not been received. [_] been filed in parent application Serial No. _____, filed on _____
6. ☐ Note the attached Examiner's Amendment
7. ☐ Note the attached Examiner Interview Summary Record, PTOL-413.
8. ☒ Note the attached Examiner's Statement of Reasons for Allowance
9. ☐ Note the attached NOTICE OF REFERENCES CITED, PTO-892
10. ☐ Note the attached INFORMATION DISCLOSURE CITATION, PTO-1449.

PART II.

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" indicated on this form. Failure to timely comply will result in the ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

1. ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.
2. ☒ APPLICANT MUST MAKE THE DRAWING CHANGES INDICATED BELOW IN THE MANNER SET FORTH ON THE REVERSE SIDE OF THIS PAPER.
   a. ☒ Drawing informalities are indicated on the NOTICE RE PATENT DRAWINGS, PTO-948, attached hereto or to Paper No. _____. CORRECTION IS REQUIRED.
   b. ☐ The proposed drawing correction filed on _____ has been approved by the examiner. CORRECTION IS REQUIRED.
   c. ☐ Approved drawing corrections are described by the examiner in the attached EXAMINER'S AMENDMENT. CORRECTION IS REQUIRED.
   d. ☒ Formal drawings are now REQUIRED.

Any response to this letter should include in the upper right hand corner, the following information from the NOTICE OF ALLOWANCE AND ISSUE FEE DUE: ISSUE BATCH NUMBER, DATE OF THE NOTICE OF ALLOWANCE, AND SERIAL NUMBER.

Attachments:
- _ Examiner's Amendment
- _ Examiner Interview Summary Record, PTOL-413
- ☒ Reasons for Allowance
- _ Notice of References Cited, PTO-892
- _ Information Disclosure Citation, PTO-1449
- _ Notice of Informal Application, PTO-152
- _ Notice re Patent Drawings, PTO-948
- _ Listing of Bonded Draftsmen
- _ Other

PTOL-37 (REV. 4-93) *

USCOMM-DC 69-37789

Serial No. 088,439

Art Unit 1102

The following is an Examiner's Statement of Reasons for Allowance: An appropriate search of the prior art failed to reveal any reference(s) explicitly teaches or fairly suggests a method for detecting and separating anions in a sample using capillary electrophoresis comprising the steps of providing a capillary filled with a carrier electrolyte, said carrier electrolyte containing a light-absorbing co-anion, heating or cooling said capillary to a target temperature in the range of from 25°C to 60°C to 60°C, introducing a sample containing one or more anions into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, and detecting said anions indirectly using a photometric detector while maintaining the temperature in said capillary to within ± 0.5°C of said target temperature, wherein said anions are detected by simultaneously monitoring said sample at two different wavelengths. An appropriate search of the prior art failed to reveal any reference(s) which explicitly teaches or fairly suggests a method for separating and detecting anions in a sample using capillary electrophoresis the steps providing a capillary filled with a carrier electrolyte, heating or cooling said capillary to a target temperature in the range of from 25°C to 60°C, introducing a sample containing one

or more anions into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, varying said target temperature as said anions migrate and detecting said anions. An appropriate search of the prior art failed to reveal any reference(s) which explicitly teaches or fairly suggests a method for separating and detecting anions in a sample using capillary electrophoresis comprising the steps of, providing a capillary filled with a carrier electrolyte and diethylenetriamine as an electrosmetic flow modifier, heating cooling said capillary to a target temperature in the range of from 25°C to 60°C, introducing a sample containing one or more anions into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, and detecting said anions. An appropriate search of the prior art failed to reveal any reference(s) which explicitly teaches or fairly suggests a method of separating and detecting anions in a sample using capillary electrophoresis comprising the steps of, providing a capillary filled with carrier electrolyte, heating or cooling said capillary to a target temperature in the range of from 20° to 60°C, introducing a sample containing one or more anions into said capillary, applying an electrical current to

said capillary under conditions causing anions in said sample to migrate and separate, and detecting said anions by simultaneously monitoring said sample at two different wavelengths while maintaining the temperature in said capillary to within +0.5°C of said target. An appropriate search of the prior art failed to reveal any reference(s) which explicitly teaches or fairly suggests a method for separating and detecting anions in a sample using capillary electrophoresis comprising the steps of providing a capillary filled with a carrier electrolyte, heating said capillary to a target temperature in the range of from 20° to 60°C, introducing a first portion of a sample containing one or more anions into said capillary, applying an electrical current to said capillary under conditions causing anions in said sample to migrate and separate, detecting said anions by simultaneously monitoring said sample at two different wavelengths, introducing a second portion of said sample into said capillary containing one or more anions, changing said temperature in said capillary, detecting said anions in said second portion, and comparing the order of election of said anions in said second portion with said first portion.

Any comments considered necessary by applicant must be submitted no later than the payment of the Issue Fee and, to

avoid processing delays, should preferably accompany the Issue Fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to J. Starsiak whose telephone number is (703) 308-1797.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0661.

J. Starsiak:rg
July 12, 1994

John Niebling
Supervisory Patent Examiner
Patent Examining Group 110

PTO FORM 948 (REV 7-92)

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

ATTACHMENT TO PAPER NUMBER

APPLICATION NUMBER 088439.

GROUP

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

THE PTO DRAFTSMEN REVIEW ALL ORIGINALLY FILED DRAWINGS REGARDLESS OF WHETHER THEY WERE DESIGNATED AS INFORMAL OR FORMAL. ADDITIONALLY, THE PATENT EXAMINER WILL ALSO REVIEW THE DRAWINGS FOR COMPLIANCE WITH THE REGULATIONS.

The drawings filed 7/7/93

A. ☐ are approved by the draftsperson.

B. ☒ are objected to by the draftsperson under 37 CFR 1.84 for the reason(s) checked below. The examiner will require submission of new, corrected drawings at the appropriate time. Corrected drawings must be submitted according to the instructions listed on the back of this Notice.

1. Paper and Ink. 37 CFR 1.84(a)

☐ Sheet(s)_____ Poor.

2. Size of Sheet and Margins. 37 CFR 1.84(b)

Acceptable Paper Sizes and Margins

	Paper Size		
Margin	8 1/2 by 14 inches	8 1/2 by 13 inches	DIN size A4 21 by 29.7 cm
Top	2 inches	1 inch	2.5 cm.
Left	1/4 inch	1/4 inch	2.5 cm.
Right	1/4 inch	1/4 inch	1.5 cm.
Bottom	1/4 inch	1/4 inch	1.0 cm.

☐ Proper Size Paper Required. All Sheets Must be Same Size. Sheet(s) _____

☒ Proper Margins Required. Sheet(s) 1-3,7

☐ TOP ☐ RIGHT

☒ LEFT ☐ BOTTOM

3. Character of Lines. 37 CFR 1.84(c)

☒ Lines Pale or Rough and Blurred. Fig(s) 1A-6

☐ Solid Black Shading Not Allowed. Fig(s) _____

4. ☐ Photographs Not Approved. _____

5. Hatching and Shading. 37 CFR 1.84(d)

☐ Shade Lines are Required Fig(s) _____

☐ Criss-Cross Hatching Not Allowed. Fig(s) _____

☐ Double Line Hatching Not Allowed. Fig(s) _____

☐ Parts in Section Must be Hatched Fig(s) _____

6. Reference Characters. 37 CFR 1.84(l)

☒ Reference Characters Poor or Incorrectly Sized. Fig(s) 1A-6

☐ Reference Characters Placed Incorrectly. Fig(s) _____

7. Views. 37 CFR 1.84(i) & (j)

☐ Figures Must be Numbered Properly.

☐ Figures Must Not be Connected. Fig(s) _____

8. ☐ Identification of Drawings. 37 CFR 1.84(l) Extraneous Matter or Copy Machine Marks Not Allowed. Fig(s) _____

9. ☐ Changes Not Completed from Prior PTO-948 dated _____

☐ Comments;

Telephone inquires concerning this review should be directed to the Chief Draftsperson at telephone number (703) 305-8404.

AD
Reviewing Draftsperson

8/13/93
Date

Note: Any objection to the drawings made by the examiner will be communicated separately in an office action.

PTO Copy

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: Box ISSUE FEE
COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

D1M1/0714

KILLWORTH, GOTTMAN, HAGAN & SCHAEFF
ONE DAYTON CENTRE
ONE SOUTH MAIN STREET, SUITE 500
DAYTON, OHIO 45402-2023

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

☒ Note attached communication from the Examiner
☐ This notice is issued in view of applicant's communication filed _____

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT		DATE MAILED
08/088,439	07/07/93	017	STARSIAK, J	1102	07/14/94

First Named Applicant: KELLY, LENORE

TITLE OF INVENTION: CONTROLLED TEMPERATURE ANION SEPARATION BY CAPILLARY ELECTROPHORESIS

	ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
1	SPA096PA	204-180.100	C63	UTILITY	NO	$1170.00	10/14/94

THE FEE DUE IS THE AMOUNT IN EFFECT AT THIS TIME. IF THE AMOUNT OF THE ISSUE FEE INCREASES PRIOR TO PAYMENT, APPLICANT WILL BE NOTIFIED OF THE BALANCE OF ISSUE FEE DUE.

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT.

PROSECUTION ON THE MERITS IS CLOSED.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.

HOW TO RESPOND TO THIS NOTICE:

I. Review the SMALL ENTITY Status shown above.
If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is changed, pay twice the amount of the FEE DUE shown above and notify the patent and Trademark Office of the change in status, or
B. If the Status is the same, pay the FEE DUE shown above.

If the SMALL ENTITY is shown as NO:
A. Pay FEE DUE shown above, or
B. File verified statement of Small Entity Status before, or with, pay of 1/2 the FEE DUE shown above.

II. Part B of this notice should be completed and returned to the Patent and Trademark Office (PTO) with your ISSUE FEE. Even if the ISSUE FEE has already been paid by charge to deposit account, Part B should be completed and returned. If you are charging the ISSUE FEE to your deposit account, Part C of this notice should also be completed and returned.

III. All communications regarding this application must give series code (or filing date) and serial number. Please direct all communications prior to issuance to Box ISSUE FEE unless advised to contrary.

IMPORTANT REMINDER: Patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (REV 7-92) (OMB Clearance is pending) PATENT AND TRADEMARK OFFICE COPY

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/088,439	07/07/93	KELLY	L SPA096PA

EXAMINER
STARSIAK, J

D1M1/0802

KILLWORTH, GOTTMAN, HAGAN & SCHAEFF
ONE DAYTON CENTRE
ONE SOUTH MAIN STREET, SUITE 500
DAYTON, OHIO 45402-2023

ART UNIT	PAPER NUMBER
1102	10

DATE MAILED: 08/02/94

SUPPLEMENTAL NOTICE OF ALLOWABILITY

PART I.

1. ☒ This communication is responsive to applicant's communication filed 5 July 1994
2. ☒ All the claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice Of Allowance And Issue Fee Due or other appropriate communication will be sent in due course.
3. ☒ The allowed claims are 1, 4-6, and 8-21
4. ☐ The drawings filed on _____ are acceptable.
5. ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has (__) been received. (__) not been received (__) been filed in parent application Serial No. _____, filed on _____
6. ☐ Note the attached Examiner's Amendment.
7. ☐ Note the attached Examiner Interview Summary Record, PTOL-413.
8. ☐ Note the attached Examiner's Statement of Reasons for Allowance.
9. ☐ Note the attached NOTICE OF REFERENCES CITED, PTO-892.
10. ☐ Note the attached INFORMATION DISCLOSURE CITATION, PTO-1449.

PART II.

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" indicated on this form. Failure to timely comply will result in the ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

1. ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.
2. ☐ APPLICANT MUST MAKE THE DRAWING CHANGES INDICATED BELOW IN THE MANNER SET FORTH ON THE REVERSE SIDE OF THIS PAPER.
   a. ☐ Drawing informalities are indicated on the NOTICE RE PATENT DRAWINGS, PTO-948, attached hereto or to Paper No. _____. CORRECTION IS REQUIRED.
   b. ☐ The proposed drawing correction filed on _____ has been approved by the examiner. CORRECTION IS REQUIRED.
   c. ☐ Approved drawing corrections are described by the examiner in the attached EXAMINER'S AMENDMENT. CORRECTION IS REQUIRED.
   d. ☐ Formal drawings are now REQUIRED.

Any response to this letter should include in the upper right hand corner, the following information from the NOTICE OF ALLOWANCE AND ISSUE FEE DUE: ISSUE BATCH NUMBER, DATE OF THE NOTICE OF ALLOWANCE, AND SERIAL NUMBER.

Attachments:
- _ Examiner's Amendment
- _ Examiner Interview Summary Record, PTOL-413
- _ Reasons for Allowance
- _ Notice of References Cited, PTO-892
- _ Information Disclosure Citation, PTO-1449
- _ Notice of Informal Application, PTO-152
- _ Notice re Patent Drawings, PTO-948
- _ Listing of Bonded Draftsmen
- _ Other

John Niebling
Supervisory Patent Examiner
Patent Examining Group 110

PTOL-37 (REV. 4-93) *

USCOMM-DC 80-3780

4100-9/6/94 #11
023-7/22 JM

(1)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of

Applicants	:	Lenore Kelly, Dean S. Burgi, Robert J. Nelson
Serial No.	:	08/088,439
Filed	:	July 7, 1993
Title	:	CONTROLLED TEMPERATURE ANION SEPARATION BY CAPILLARY ELECTROPHORESIS
Docket	:	SPA 096 PA
Art Unit	:	1102
Examiner	:	J. Starsiak

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on September 15, 1994

Timothy W. Hagan 29,001
Attorney Reg. No.

Hon. Commissioner of Patents
and Trademarks
Washington, DC 20231

Attn: Official Draftsperson

Sir or Madam:

TRANSMITTAL OF FORMAL DRAWINGS

Enclosed herewith are formal drawings for the above-identified patent application.

Respectfully submitted,

KILLWORTH, GOTTMAN, HAGAN & SCHAEFF

By Timothy W. Hagan
Timothy W. Hagan
Registration No. 29,001

One Dayton Centre
One South Main Street, Suite 500
Dayton, Ohio 45402-2023
Telephone: (513) 223-2050
Facsimile: (513) 223-0724

TWH:lrl
Encls.

09/088,439

5385654

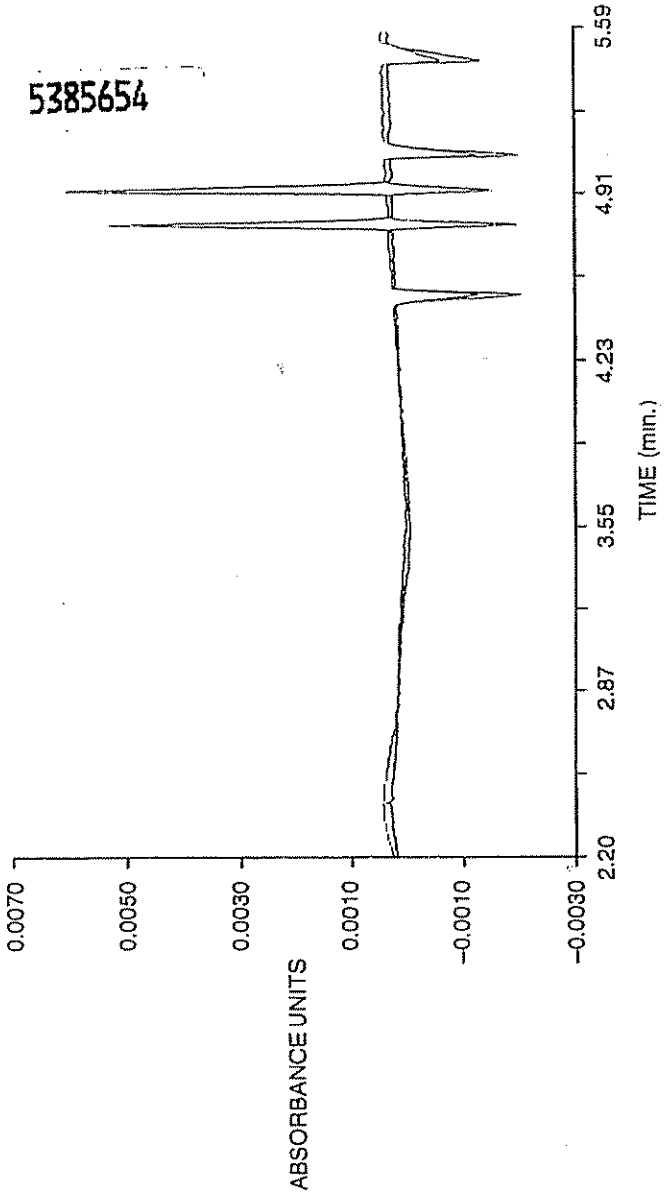


FIG-1A

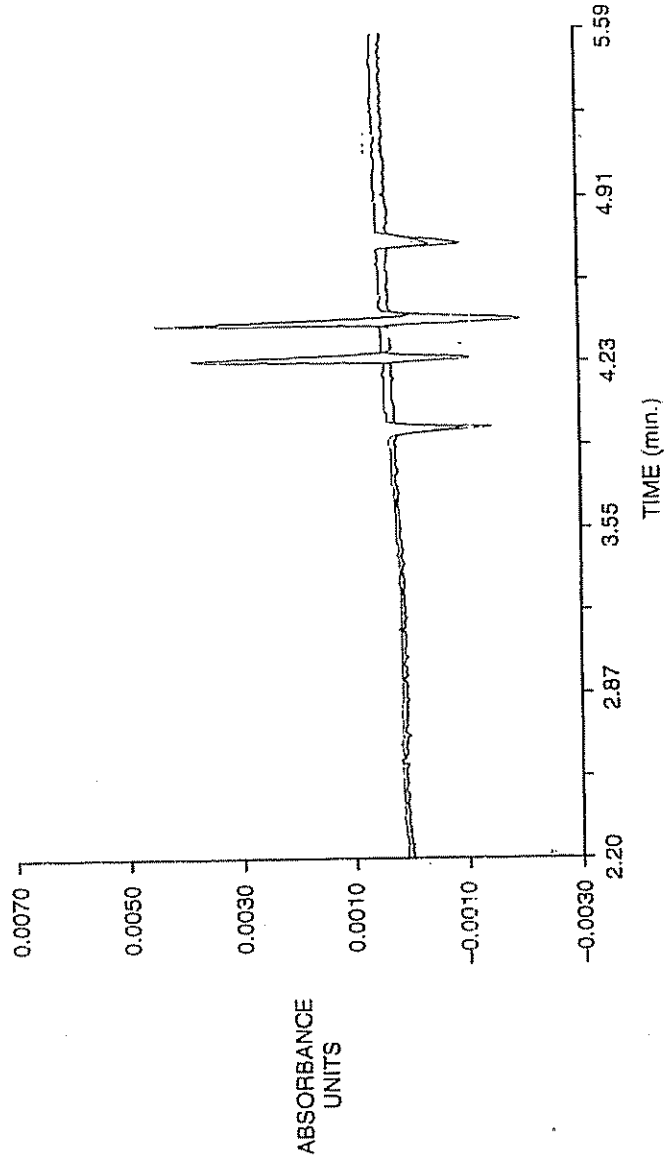

0.0070
0.0050
0.0030
0.0010
-0.0010
-0.0030
ABSORBANCE UNITS
2.20
2.87
3.55
4.23
4.91
5.59
TIME (min.)

FIG-1B

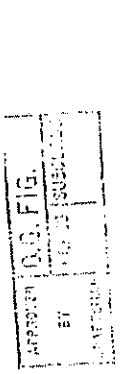
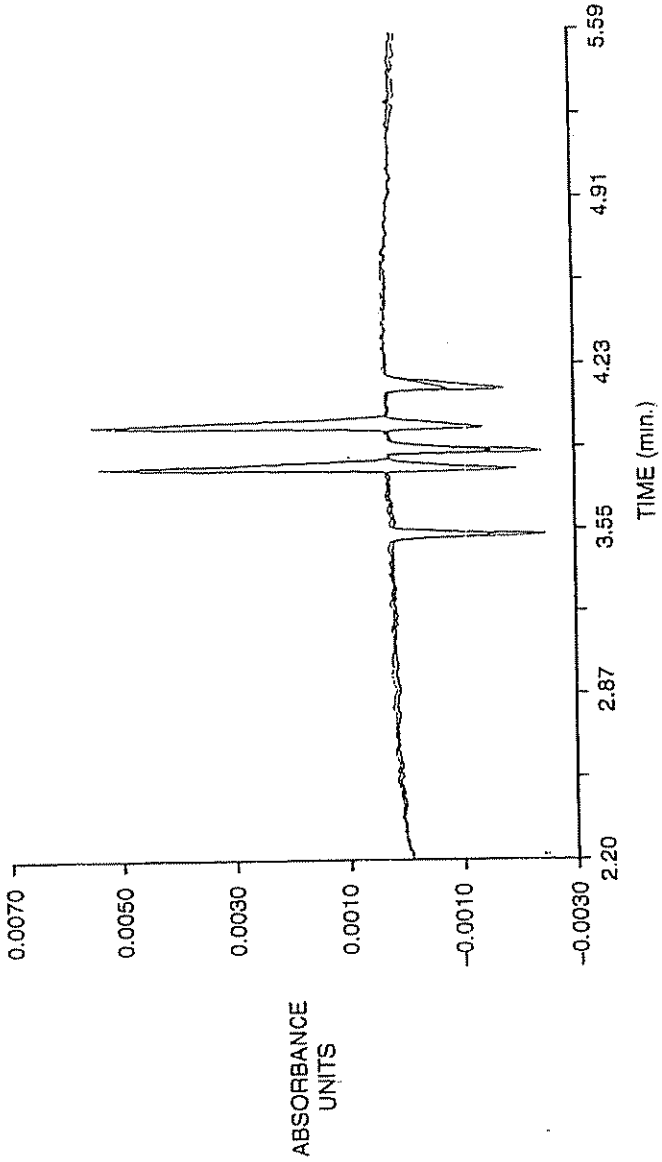


FIG-1C


0.0070
0.0050
0.0030
0.0010
−0.0010
−0.0030
ABSORBANCE UNITS
2.20
2.87
3.55
4.23
4.91
5.59
TIME (min.)

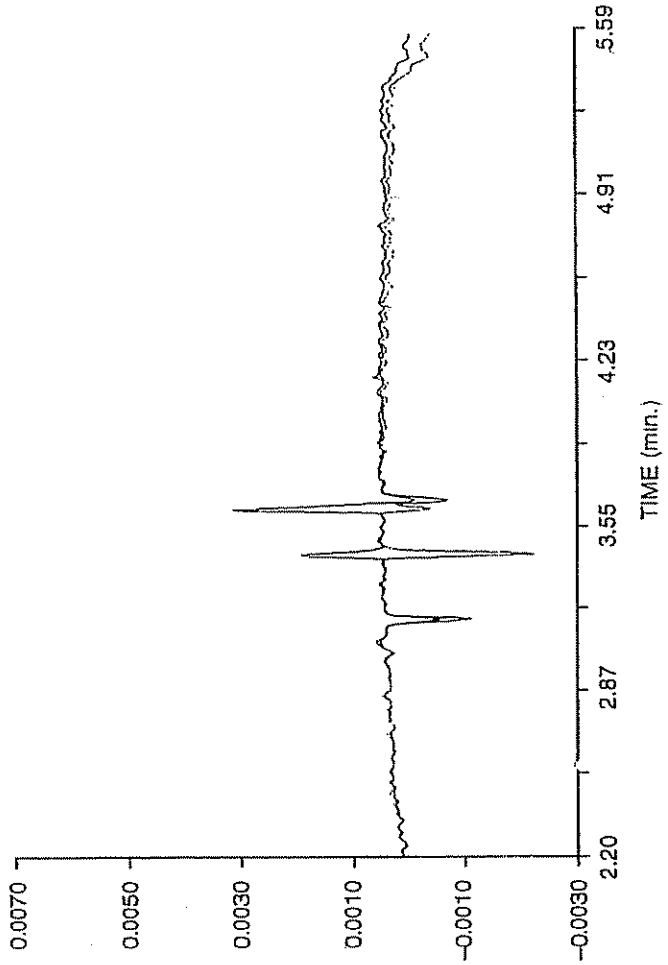

0.0070
0.0050
0.0030
0.0010
-0.0010
-0.0030
ABSORBANCE UNITS
2.20
2.87
3.55
4.23
4.91
5.59
TIME (min.)

FIG-1D

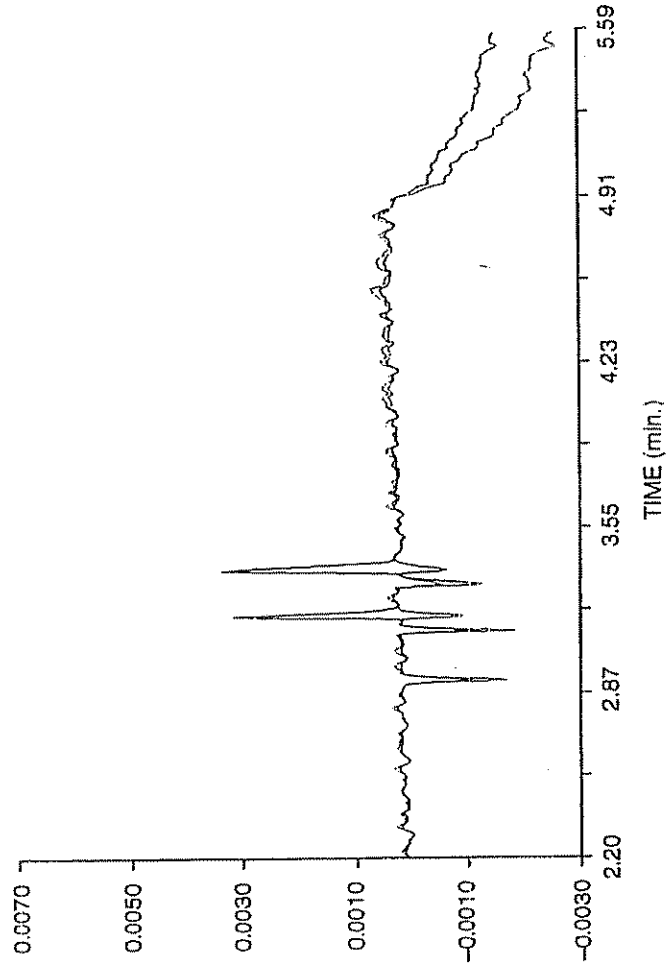
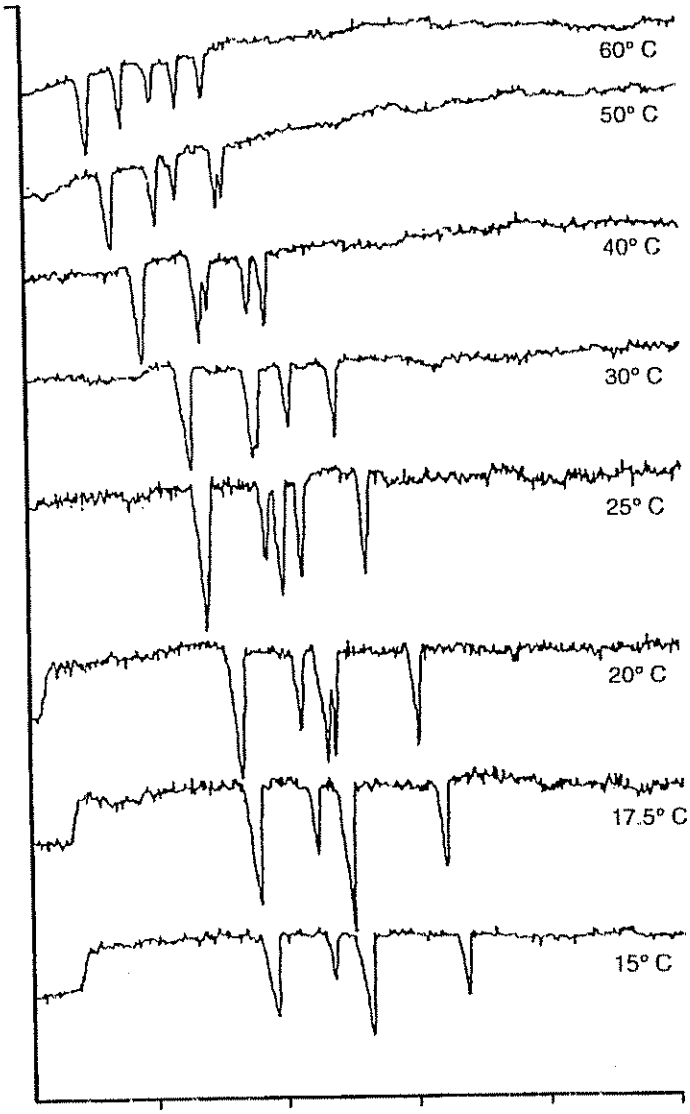


FIG-1E

FIG-2



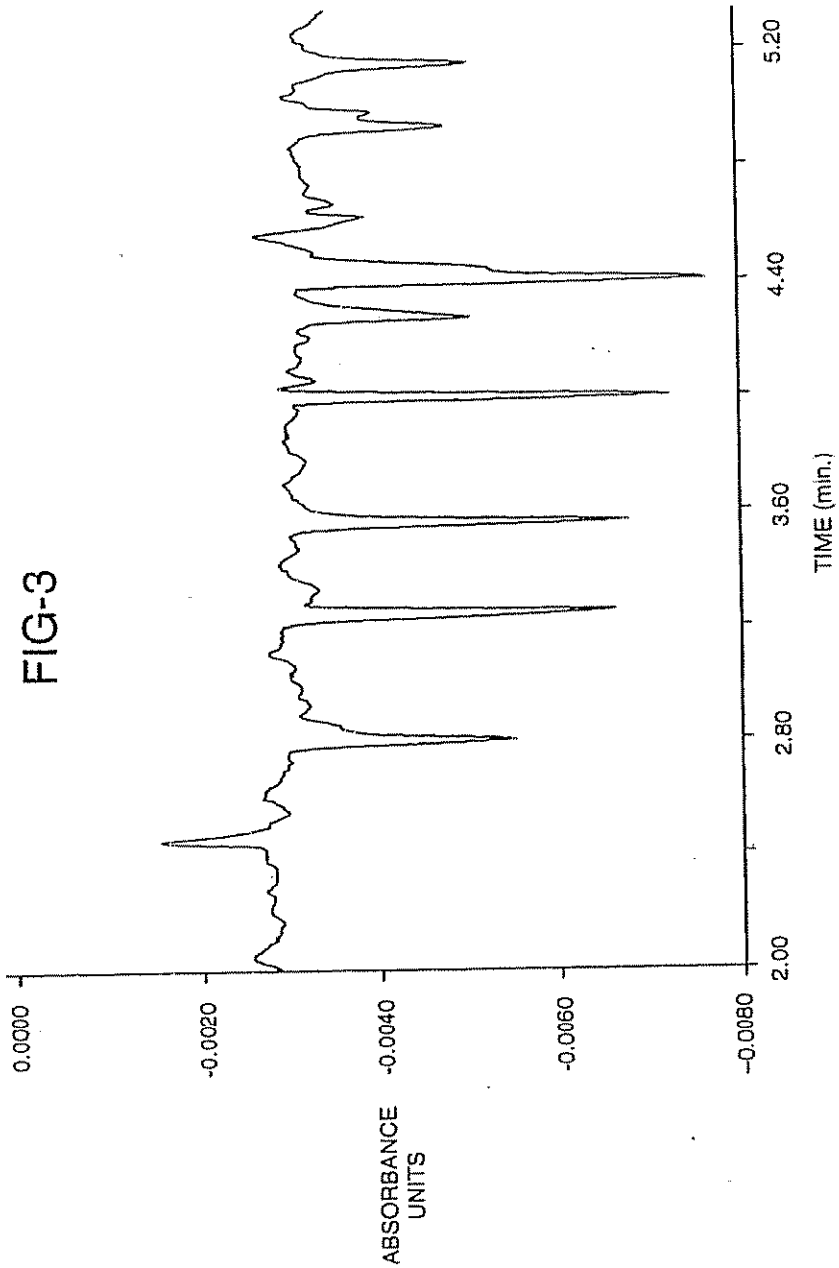
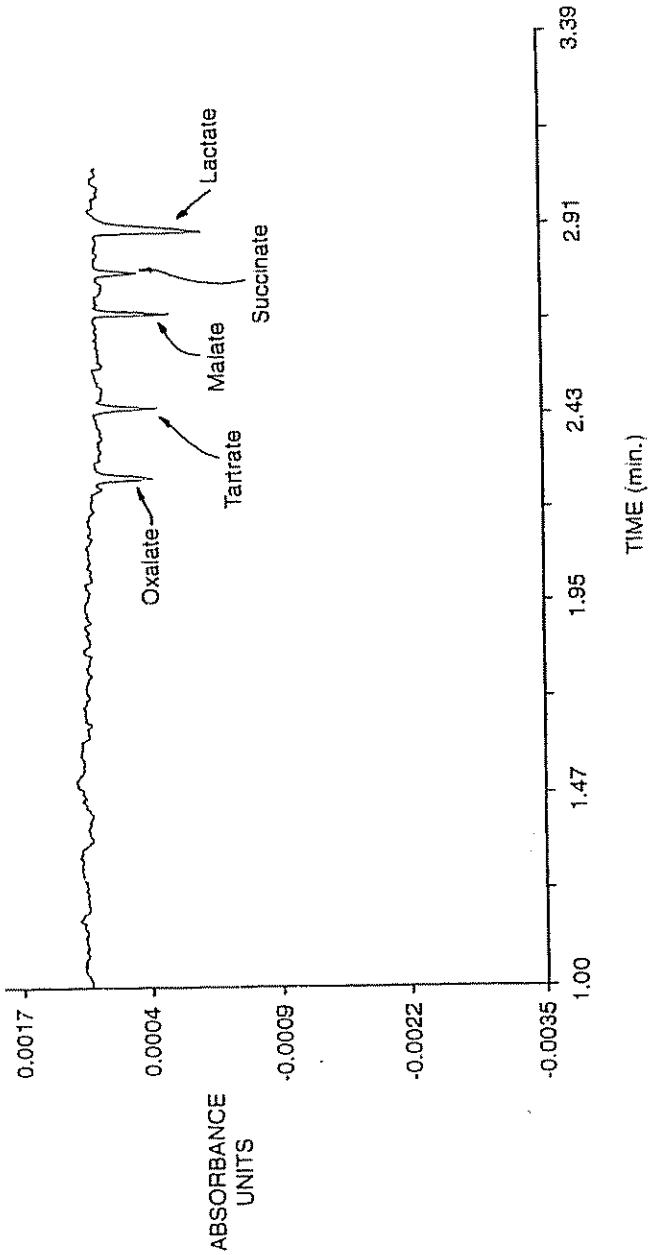

0.0000
-0.0020
-0.0040
-0.0060
-0.0080
ABSORBANCE UNITS
2.00
2.80
3.60
4.40
5.20
TIME (min.)

FIG-3

FIG-4



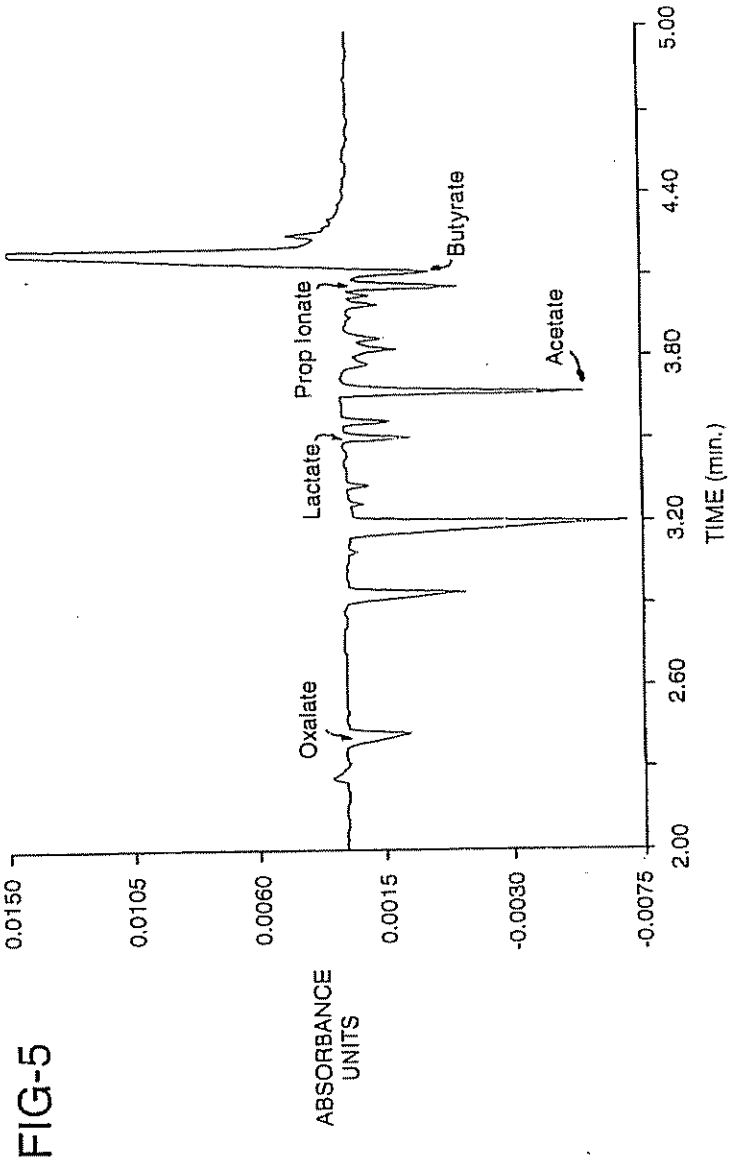

Oxalate
Lactate
Prop Ionate
Butyrate
Acetate
0.0150
0.0105
0.0060
0.0015
-0.0030
-0.0075
ABSORBANCE UNITS
2.00
2.60
3.20
3.80
4.40
5.00
TIME (min.)

FIG-5

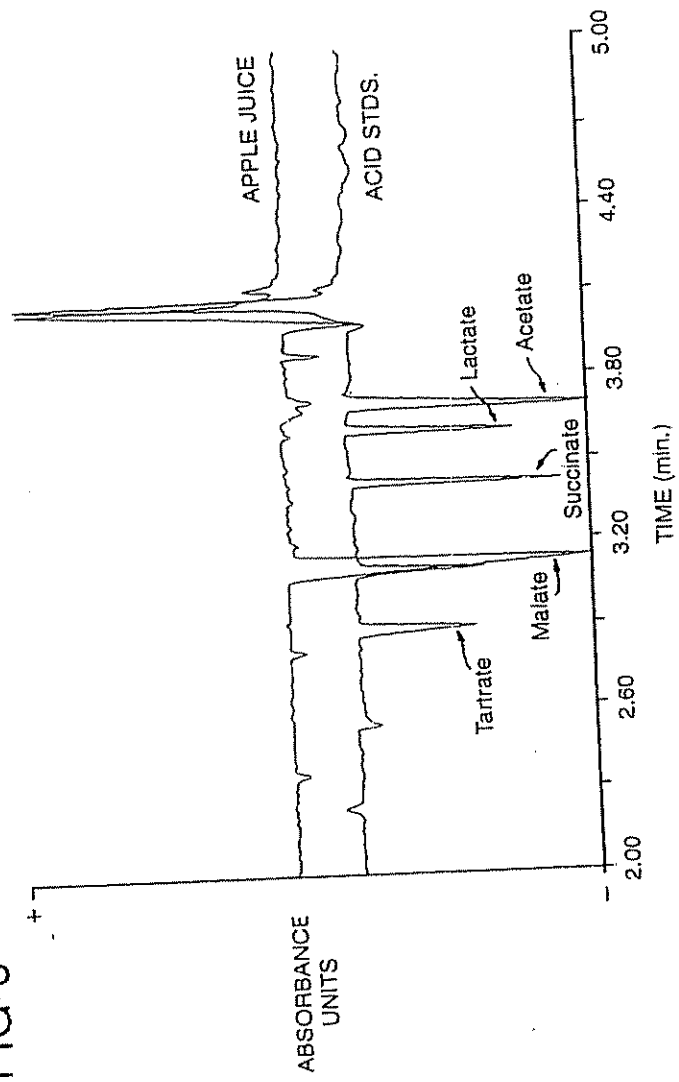

APPLE JUICE
ACID STDS.
Lactate
Acetate
Succinate
Malate
Tartrate
5.00
4.40
3.80
3.20
2.60
2.00
TIME (min.)
+
-
ABSORBANCE UNITS

FIG-6

DEAR CLIENT:

THIS IS THE BEST COPY AVAILABLE, OF THE ATTACHED PAGE(S), DUE TO THE CONDITION OF THE SOURCE DOCUMENT.

PLEASE BE ASSURED THAT EVERY EFFORT HAS BEEN MADE TO SUPPLY YOU WITH THE HIGHEST QUALITY DOCUMENTATION

Patent Imaging Corporation
Patent Legal and Scientific Information Service
2001 Jefferson Davis Highway
Crystal Plaza One, Suite 600
Arlington, VA 22202-3610
(703) 553-0000

PART B—ISSUE FEE TRANSMITTAL

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE. Blocks 2 through 6 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advances orders and notification of maintenance fees will be mailed to the address entered in Block 1 unless directed otherwise, by: (a) specifying a new correspondence address in Block below; or (b) providing the PTO with a separate "FEE ADDRESS" for maintenance notifications with the payment of Issue Fee or thereafter. See reverse for Certificate of Mailing.

MAIL ROOM OCT 6 1994 PAT. & TRADEMARK OFF.

1. CORRESPONDENCE ADDRESS

KILLWORTH, GOTTMAN, HAGAN & SCHAEFF
ONE DAYTON CENTRE
ONE SOUTH MAIN STREET, SUITE 500
DAYTON, OHIO 45402-2023

2. INVENTOR(S) ADDRESS CHANGE (Complete only if there is a change)

INVENTOR'S NAME
(Street Address)
City, State and ZIP Code

CO-INVENTOR'S NAME
Street Address
City, State and ZIP Code

☐ Check if additional changes are on reverse side

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
08/088,439	07/07/93	017	STARSIAK, J. 1102	07/14/94

First Named Applicant: KELLY, LENORE

TITLE OF INVENTION: CONTROLLED TEMPERATURE ANIONIC SEPARATIONS BY CAPILLARY ELECTROPHORESIS

	ATTY'S DOCKET NO.	CLASS/SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
1	SFA096PA	204-180.100	C63	UTILITY	NO	$1170.00	10/14/94

7. Correspondence address change (Complete only if there is a change)

4. For printing on the patent front page, list the names of not more than 3 registered patent attorneys or agents OR alternatively, the name of a firm having as a member a registered attorney or agent. If no name is listed, no name will be printed

1 Killworth,
2 Gottman, Hagan &
3 Schaeff

OBO WE 10/06/94 08088439 DO NOT USE THIS SPACE 1170.00 CK

5. ASSIGNMENT DATA TO BE PRINTED ON THE PATENT (print or type)

(1) NAME OF ASSIGNEE: Thermo Separation Products, Inc.

(2) ADDRESS: (CITY & STATE OR COUNTY) Fremont, California

(3) STATE OF INCORPORATION, IF ASSIGNEE IS A CORPORATION: Delaware

A. ☐ This application is NOT assigned.
☒ Assignment is being previously submitted to the Patent and Trademark Office.
☐ Assignment is being submitted under separate cover. Assignments should be directed to Box ASSIGNMENTS.
PLEASE NOTE: Unless an assignee is identified in Block 5, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

6a. The following fees are enclosed:
☒ Issue Fee ☐ Advanced Order - # of Copies ____ (Minimum of 10)

6b. The following fees should be charged to:
DEPOSIT ACCOUNT NUMBER ____
(ENCLOSED PART C)
☐ Issue Fee ☐ Advanced Order - # of Copies ____ (Minimum of 10)
☐ Any Deficiencies In Enclosed Fees

The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee to the application identified above.

(Signature of party indicated at paragraph A) (Date)
Timothy M Hagan Reg. No. 29001 9/30/94

NOTE: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

TRANSMIT THIS FORM WITH FEE-CERTIFICATE OF MAILING ON REVERSE

PTOL 85B (REV 7-92)(OMB Clearance is pending)

PTO UTILITY GRANT

Paper Number 12

The United States of America

The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

Bruce Lehman

Commissioner of Patents and Trademarks

Marjorie V. Jones

Attest

PTO-1584

(RIGHT INSIDE)

CHECKLIST FOR ISSUE REVISION

0,8 3 73/

Instructions

Revise the entire Application before returning it to the SPE for correction.
If corrections are required, list them in the sections provided at the end of the checklist.
Make a checkmark beside item if present and correct

THE CENTER SECTION IS ARRANGED AS FOLLOWS (TOP TO BOTTOM):

I. DRAWINGS

__1. None and agrees with drawing box face of file (if checked go to B. PCT Data).
__2. There are no cancellation stamps, pencil or RED lines.
__3. Serial number is correct on each sheet of drawings.
__4. Drafting Stamp is on each sheet of drawing.
__5. Drafting Stamp is on sheet one contains class/subclass and figure of drawing for printing in Official Gazette.
__6. Drafting Stamp on sheet where figure for printing is located contains class/subclass and figure of drawing for printing in Official Gazette.
__7. Yellow tag present for drawing correction.
__8. PTOL 37 is present.
__9. PTOL 37 has been completed to notify applicant of drawing requirement.
__10. Official Gazette print figure box (on face of file) complete and correct.
__11. Number of sheets in file agrees with drawing box on face of file.
__12. Number of figures of drawings agrees with box on face of file (if the number is more than one, write number of figures in lower right hand corner on first sheet.
__13. Brief Description of Drawings includes a description for each figure of drawing.

PCT DATA (IF FILED UNDER 35 USC 371)

__1. Filed under 35 USC 371 (if not checked go to C PTO Forms).
__2. PCT Form 903 present.
__3. PCT Gazette page present.

PTO-FORM(S) 892 AND 1449

__1. At least one form is in file.
__2. Serial number is present and correct on each form
__3. U.S. entries include patent number, name of inference, month and year.
__4. Foreign entries include publication number, country, month and year.
__5. Class and subclass boxes have classification entered or lined through.
__6. Examiner signed and dated.
__7. Examiner initialed or lined through entries on all PTO 1449's.

AMENDMENTS

__1. None (if checked, go to E Abstract)
__2. Each amendment listed in the "CONTENTS" of file.
__3. Amendments has been entered as instructed.
__4. All written entries are legible.
__5. Pencil notations erased.
__6. Examiner Amendments signed by primary examiner or SPE, entered made of record and mailed.
__7. Amendments without boxed inserts have been moved to right side of file.
__8. Claims have been renumbered as indicated by examiner.

E. ABSTRACT

__1. Abstract on separate page.
__2. Serial Number present and correct.
__3. Contains no more than 25 lines.
__4. No more than one paragraph.

F. SPECIFICATION

__1. Only one specification present.
__2. Specification is in permanent ink.
__3. Serial number is present and correct on first page.
__4. Continuing data is mentioned in the first paragraph or on insert before.
__5. Continuing data has been updated.
__6. Continuing data agrees with face of file.
__7. There are no unclear words because of holes at top of any page.
__8. There is no missing text.
__9. There are no missing or duplicated pages.
__10. Pencil notations erased.

G. CLAIMS

__1. All claims are either renumbered or cancelled.
__2. There are no missing or duplicated claim numbers.
__3. Claims, including dependency are renumbered in RED ink where necessary.
__4. Total number in "INDEX of CLAIMS" corresponds with Claims Allowed.
__5. Total number in "INDEX of CLAIMS" corresponds with PTOL 37 and 85.

H. OATH/DECLARATION

__1. Declaration Statement is included.
__2. Oath (notary seal is present).
__3. All phrase are included:
  __a. "The original and first inventor or inventors".
  __b. "Reviewed and understand the contents of the specification including claims".
  __c. "Acknowledge the duty to disclose information in accordance with 1.56(a)".
__4. Signed by all applicants or Rule 47 label attached.
__5. Executed no more than 3 months before filing date.
__6. Residence, citizenship and Post Office address included for all applicants.
__7. Foreign priority claimed no more than one year from effective filing date (six months for designs).
__8. Foreign priority on face of file agrees with Oath/Declaration.
__9. Examiner has checked and initialed foreign data boxes.
__10. Certified copy of foreign priority document present.
__11. Examiner has acknowledged foreign priority.

I. SMALL ENTITY

___1. Small Entity Statement

___2. Small Entity recorded on face of file.

___3 Small Entity entered in PALM.

J. REFERENCES

___1 None present.

___2 Publications and Foreign References (move to bottom right side).

II LEFT SIDE OF FILEWRAPPER

ISSUE CLASSIFICATION SLIP

___1. Issue Classification Slip affixed to Staple Area.

___2. Serial Number present and correct.

___3. Applicant's name present and correct.

___4. Issue Classification Slip class and subclass correspond with notation on drafting stamp (if any) and Issue Classification Box on face of file.

___5. International classification has been completed.

___6. Signed by a Primary Examiner.

___a. Signed by an Assistant Examiner.

___b. Assistant Examiner box lined through.

III RIGHT SIDE OF FILEWRAPPER IS ARRANGED FOLLOWS (TOP TO BOTTOM).

___ Notice of Allowance (PTOL 85)

___ Papers with paper numbers arranged in descending paper number order (most rec paper on top)

___ Fee Determination Record.

___ PALM Coding Sheet.

___ Papers with dates arranged in descending date order (most recent paper on top).

___ Papers without dates.

___1 Documents arranged in order.

___2. All documents are liste[d] . CONTENTS" [i]front according to date a[nd] checked off in colored pencil.

___3. Notice of Allowance title, class, subclass, number of claims correspond with face of file.

IV. RIGHT OUTSIDE FILEWRAPPER

___1. Searched Box is completed.

___2. Interference Search Box is completed.

___3. An issue fee authorization stamp is present

V. FACE OF FILE

___1. Title changes are in RED.

___2. Signed by a Primary Examiner.

___a. Signed by an Assistant Examiner.

___b. Assistant Examiner box lined throu[gh]

___3. Print claim box completed.

___4. Terminal disclaimer sticker includes date.

___5. Allowance boxes completed (Notice of Allowance mailed, Amount Due, Issue Batch Number).

___6. Application Examiner signs and dates if no corrections are needed.

IV NOTES

Examiner, please make the following corrections:

1. You have 17 claims
2. there are 18 claim 4
3. has not been cancel
4. Or re-numbered.
5.
6.

Supervisor, please correct the following errors.

1
2.
3.
4.
5.

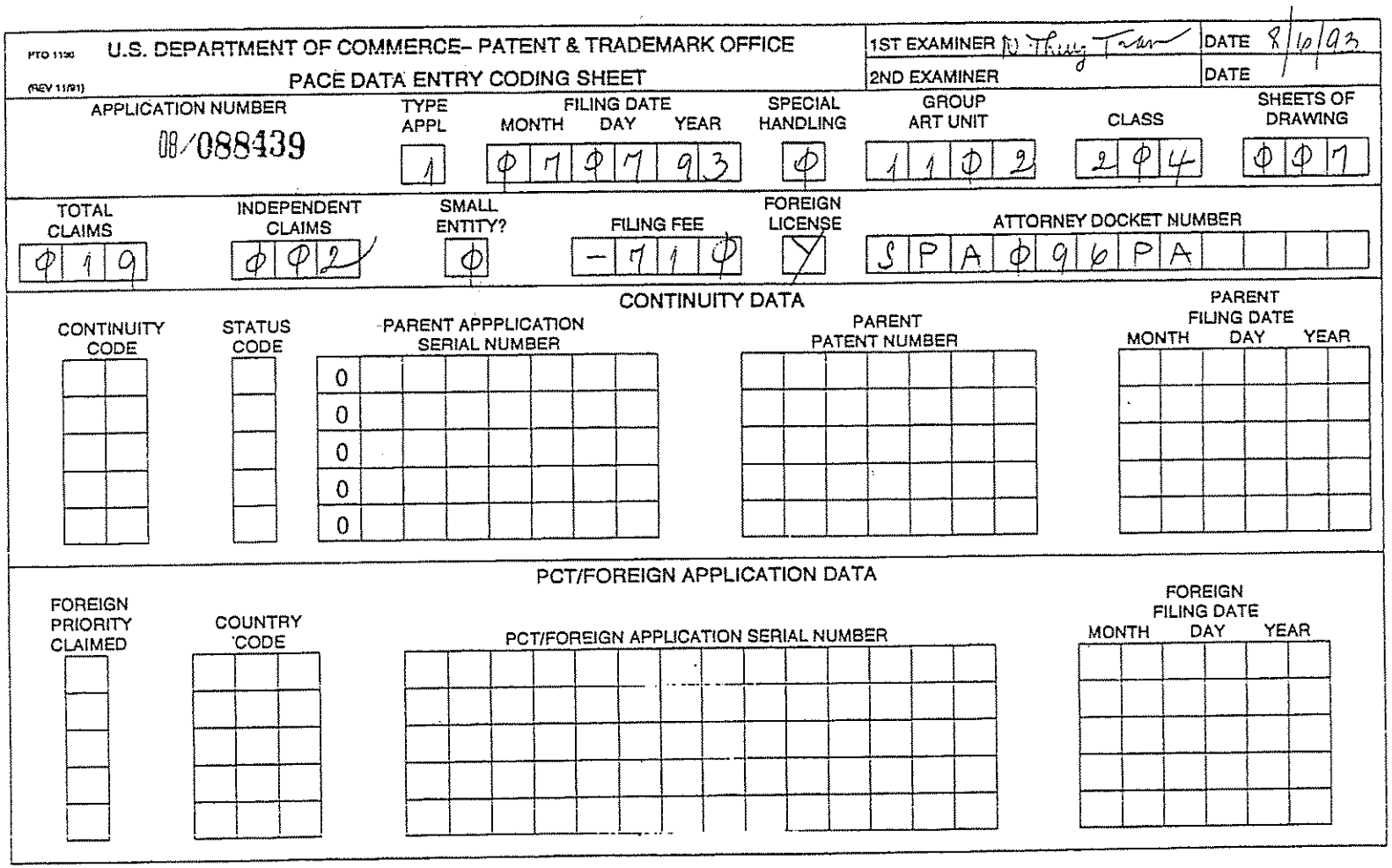
Notes to Yourself:

1.
2.
3.

RETURN TO Marsha AFTER CORRECTIONS HAVE BEEN MADE.

II. SIGNATURE [signature] DATE 7/26

SIGN ONLY WHEN NO CORRECTIONS ARE REQUIRED OR CORRECTIONS HAVE BEEN MADE.



PTO 1130 (REV 11/91)

U.S. DEPARTMENT OF COMMERCE– PATENT & TRADEMARK OFFICE

PACE DATA ENTRY CODING SHEET

1ST EXAMINER D. Thuy Tran — DATE 8/10/93

2ND EXAMINER — DATE

APPLICATION NUMBER	TYPE APPL	FILING DATE MONTH	DAY	YEAR	SPECIAL HANDLING	GROUP ART UNIT	CLASS	SHEETS OF DRAWING
08/088439	1	07	09	93	0	1102	204	007

TOTAL CLAIMS	INDEPENDENT CLAIMS	SMALL ENTITY?	FILING FEE	FOREIGN LICENSE	ATTORNEY DOCKET NUMBER
019	002	0	-710	Y	SPA0 96PA

CONTINUITY DATA

CONTINUITY CODE	STATUS CODE	PARENT APPPLICATION SERIAL NUMBER	PARENT PATENT NUMBER	PARENT FILING DATE MONTH	DAY	YEAR
		0				
		0				
		0				
		0				
		0				

PCT/FOREIGN APPLICATION DATA

FOREIGN PRIORITY CLAIMED	COUNTRY CODE	PCT/FOREIGN APPLICATION SERIAL NUMBER	FOREIGN FILING DATE MONTH	DAY	YEAR

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1992

Application or Docket Number: 08/088439

CLAIMS AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
BASIC FEE				$355.00	OR		$710.00
TOTAL CLAIMS	19 minus 20 =	*	x$11=		OR	x$22=	
INDEPENDENT CLAIMS	2 minus 3 =	*	x 37=		OR	x 74=	
MULTIPLE DEPENDENT CLAIM PRESENT			+115=		OR	+230=	
			TOTAL		OR	TOTAL	710

* If the difference in column 1 is less than zero, enter "0" in column 2

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 19	Minus	** 20	= 0
Independent	* 5	Minus	*** 3	= 2
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
x$11=		OR	x$22=	
x 37=		OR	x 74=	$148.00
+ 115=		OR	+230=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	148

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
x$11=		OR	x$22=	
x 37=		OR	x 74=	
+ 115=		OR	+ 230=	
TOTAL ADDIT FEE		OR	TOTAL ADDIT FEE	

AMENDMENT C

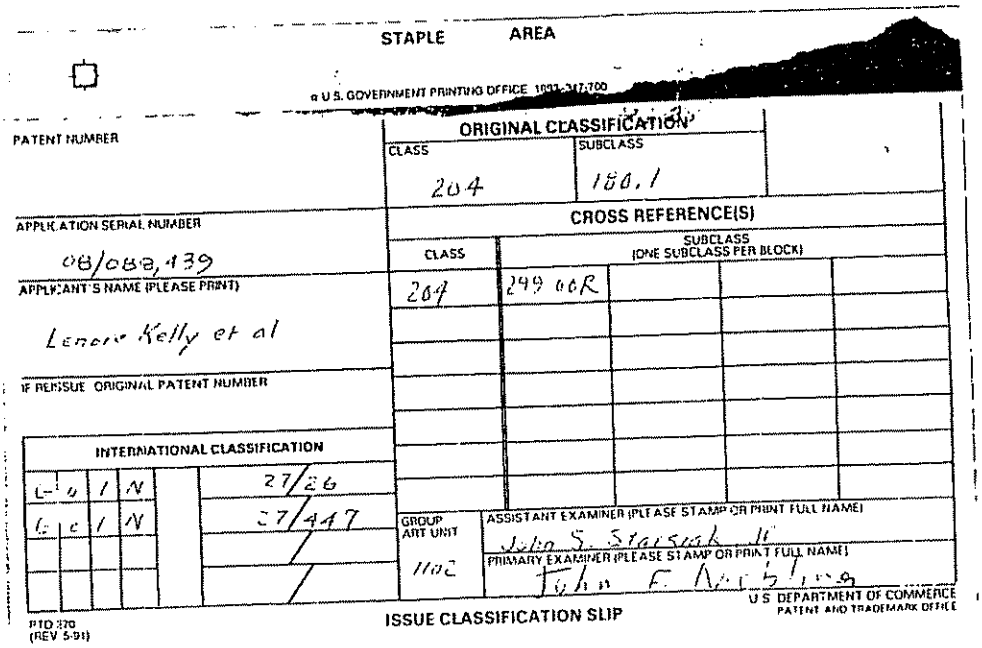
	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
x$11=		OR	x$22=	
x 37=		OR	x 74=	
+115=		OR	+230=	
TOTAL ADDIT FEE		OR	TOTAL ADDIT FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

FORM PTO-875 (Rev.10-92)

Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE



STAPLE AREA

☐

a U.S. GOVERNMENT PRINTING OFFICE 1993-347-700

PATENT NUMBER

ORIGINAL CLASSIFICATION

CLASS	SUBCLASS
204	180.1

APPLICATION SERIAL NUMBER

08/088,139

CROSS REFERENCE(S)

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)			
204	249 OCR			

APPLICANT'S NAME (PLEASE PRINT)

Lenore Kelly et al

IF REISSUE ORIGINAL PATENT NUMBER

INTERNATIONAL CLASSIFICATION

C	1	N	27/26
C	1	N	27/447
			/
			/

GROUP ART UNIT: 1102

ASSISTANT EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

Julia S. Starsak II

PRIMARY EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

John F. Kerr 5/1/95

PTO 270 (REV 5-91)

ISSUE CLASSIFICATION SLIP

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

[redacted]

SEARCHED

Class	Sub.	Date	Exmr.
204	299R 180.1	9/23/97 "	JSS "
SEARCH	UPDATED	1/28/98	JSS
SEARCH	UPDATED	7/11/98	JSS

35

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.
204 "	299E (90.1	7/11/98 "	JSS "

SEARCH NOTES

	Date	Exmr.

(RIGHT OUTSIDE)

Staple Issue Slip Here

POSITION		ID NO.	DATE
CLASSIFIER		7	7-30-93
EXAMINER		434	8/6/93
TYPIST		324	8/11/93
VERIFIER		106	8-13-93
CORPS CORR.			
SPEC. HAND			
FILE MAINT.			
DRAFTING			

INDEX OF CLAIMS

Claim Final	Claim Original	Date							
		8/6/93	8/13/93	8/11/93					
1	(1)	✓	✓	=					
	2	✓							
	3	✓							
2	4	✓	✓	=					
18	5	D	=	=					
3	6	✓	✓	=					
	7	✓	✓						
4	8	D	=	=					
5	9	✓	✓	✓					
10	(10)	D	=						
6	11	✓	✓						
7	12	✓	✓						
8	13	✓	✓						
11	14	✓	✓						
12	15	✓	✓						
13	16	D	D						
15	17	✓	✓						
16	18	D	D						
17	(19)	D	=						
9	20		✓	✓					
14	21		✓	=					
	22								
	23								
	24								
	25								
	26								
	27								
	28								
	29								
	30								
	31								
	32								
	33								
	34								
	35								
	36								
	37								
	38								
	39								
	40								
	41								
	42								
	43								
	44								
	45								
	46								
	47								
	48								
	49								
	50								

SYMBOLS

- ✓ ..... Rejected
- = ... Allowed
- . (Through numeral) Canceled
- + ... Restricted
- N ..... Non-elected
- I ..... Interference
- A ..... Appeal
- O ..... Objected

Claim Final	Claim Original	Date							
	51								
	52								
	53								
	54								
	55								
	56								
	57								
	58								
	59								
	60								
	61								
	62								
	63								
	64								
	65								
	66								
	67								
	68								
	69								
	70								
	71								
	72								
	73								
	74								
	75								
	76								
	77								
	78								
	79								
	80								
	81								
	82								
	83								
	84								
	85								
	86								
	87								
	88								
	89								
	90								
	91								
	92								
	93								
	94								
	95								
	96								
	97								
	98								
	99								
	100								

(LEFT INSIDE)

08/088439

[redacted]

APPROVED FOR ENTRY
AOG
INITIALS:

Date Entered or Counted

CONTENTS

Date Received or Mailed

GROUP 115

	1.	Application papers. PRINTS. ✓	
9/29	2.	Rejection. (3)	10/20/93
	3.	Prior Art	10/13/93
	4.	Amdt. A	1/21/94 cm
	5.	Declaration	1/21/94
	6.	Change of Address	2-18-94
5/2	7.	Final Rej. (3)	5-6-94
	8.	Amdt. B (after) (2)	7-5-94
7/13	9.	Notice of Allowance	7-14-94
	10.	Suppl. Notice of Allow	8-2-94
9/20/94	11.	Formal Drawings (10 sht(s) set 1 PTOGRANT JAN 31 1995	9/9/94
	12.		
	13.		
	14.		
	15.		
	16.		
	17.		
	18.		
	19.		
	20.		
	21.		
	22.		
	23.		
	24.		
	25.		
	26.		
	27.		
	28.		
	29.		
	30.		
	31.		
	32.		

(FRONT)